1. INTRODUCTION

1.1. Iontophoresis in Drug Delivery

1.1.1. History

Clinical application of electricity can be traced back to the ancient time of the Golden Age of the Greek Civilization. Iontophoresis originates from the Greek language and could be described as a procedure of facilitating movement of charged molecules across tissue borders under an externally applied electrical field. Iontophoresis has been used in the field of medicine for many years. One of the scientists who marked the establishment of iontophoresis in medicine was Leduc [1]. During the early twentieth century he was conducting the experiments that established the usefulness of this technique in drug delivery. He demonstrated that rabbits suffered fatal seizures minutes after transcutaneous iontophoretic administration of strychnine. Numerous studies of iontophoresis application were performed in several fields of medicine in subsequent decades. Gibson and Cooke who studied this technique in 1959 demonstrated an induction of sweating by iontophoretic application of pilocarpine [2]. Further studies analyzed the use of iontophoresis for delivering steroids in the treatment of muscoloskeletal (e.g. rheumatoid arthritis, osteoarthritis, bursitis) and urological conditions (e.g. Peyronie's disease) [3-4].

More recently, iontophoresis was studied in otolaryngology for administration of local anesthesia to the tympanic membrane [5] and vidarabine monophosphate for the treatment of herpes simplex orolabialis [6] or in the administration of gentamicin for the management of burned ears [7]. The use of iontophoresis in the field of dentistry was extensively studied by Gangarosa and his co-workers [8]. Further studies were published in dermatology during the eighties [9].

It was the German investigator Wirtz who employed iontophoresis in ophthalmology as early as 1908 [10]. He passed electric current through electrolyte –saturated cotton sponges placed over the eye globe for the treatment of conditions such as corneal ulcers, keratitis, and episcleritis. By the turn of the of the last century iontophoresis was extensively studied by a number of European investigators [11] such as Birkhauser [12], Fietta [13], Morisot [14] for a wide array of ophthalmologic conditions like corneal leukoma, recalcitrant posterior synechiae, scleritis, glaucoma, cataract or optic atrophy. Thanks to the work of Erlanger [15] this technique was introduced in England and the USA. By 1950, iontophoresis was studied intensively in England, Canada and the United States [16-19]. Witzel and colleagues [20] investigated iontophoresis as an aqueous drug delivery system for a variety of antibiotics (e.g., tetracyclines, chloramphenicol, penicillin, streptomycin, neomycin, bacitracin) in a rabbit model. In the USA one of the main investigators was Ludwig von Sallmann who published numerous reports throughout 1940 investigating the efficacy of this procedure [21-27]. Though studied all over the world during the

first half of the century [27-28] the usefulness of iontophoresis in ophthalmology seemed to be overestimated. Many of the published studies lacked carefully controlled trials and details on toxicity. Furthermore the technique was limited by the technological development of the devices and ocular electrodes. This resulted in this technique disappearing from limelight, and it was never adopted as a standard procedure in ophthalmology [29]. It was not before the 1980 that the iontophoresis underwent something like a renaissance. Hughes and Maurice [30], who demonstrated an increased penetration of gentamicin and fluorescein by the application of iontophoresis to rabbit eyes, contributed to the revival of this procedure in ophthalmology. Extensive studies with various drug applications followed.

Based on the current enthusiasm and available literature from studies performed in different fields of medicine, it may be appropriate to state that this technique holds great promise for delivering a variety of compounds for different indications, and could be effective in delivery of proteins, peptides, genes and viruses.

Table 1. Ocular Iontophoresis drug application studies since the eighties.

DRUG	LOCATION	ANIMAL	AUTHOR/YEAR
STEROIDS			
Dexamethasone	transscleral	rabbit	Lam/1989 [50]
	transcorneo-scleral	rat	Behar-Cohen/1997 [51]
Methyl-prednisolone	transscleral	rabbit	Behar-Cohen/2002 [52]
NON-STEROIDAL			
Aspirin	transscleral	rabbit	Voigt/2002 [53]
ANTIMETABOLITE			
5-Fluorouracil	transscleral	rabbit	Kondo/1989 [54]
AMINOACID-ANALOGON			
L-NAME	transcorneoscleral	rat	Behar-Cohen/1998 [55]

DRUG	LOCATION	ANIMAL	AUTHOR/YEAR
GENES			
S-ODNs	transcorneal	rabbit	Asahara/1999 [56]
ODNs	transcorneo-scleral	rat	Voigt/2002 [57]
ANTIBIOTICS			
Gentamicin	transcorneal	rabbit	Fishman/1984 [31]
	transcorneal		Hughes/1984 [30]
	transscleral		Burstein/1985 [32]
	transscleral		Grossman/1990 [33]
	transcorneal		
	transscleral		Barza/1986 [34]
Gentamicin	transscleral	monkey	Barza/1987 [35]
Gentamicin	transcorneal	rat	Frucht-Pery/1996 [36]
Cephazolin	transscleral	rabbit	Barza/1986 [34]
Ticarcillin	transscleral	rabbit	Barza/1986 [34]
Tobramycin	transcorneal	rabbit	Rootman/1988 [37]
	transcorneal	rabbit	Hobden/1988 [38]
Vancomycin	transcorneal	rabbit	Choi/1988 [39]
	transscleral		
Ciprofloxacin	transscleral	rabbit	Yoshizumi/1991 [40]
	transcorneal	rabbit	Hobden/1990 [41]
ANTIVIRAL			
Foscarnet	transscleral	rabbit	Sarraf/1993 [42]
			Yoshizumi/1996 [43]
Vidarabine	transcorneal	rabbit	Hill/1978 [44]
Ganciclovir	transscleral	rabbit	Lam/1994 [45]
	transscleral	rabbit	Chapon/1999 [46]
Acyclovir	transcorneal	rabbit	Hill/1982 [47]
ANTIFUNGAL			
Ketonconazole	transcorneal transscleral	rabbit	Grossman/1990 [48]
	transscleral	rabbit	Grossman/1989 [49]

1.1.2. Basis

Surface tissues such as skin or ocular epithelium consist of membrane barriers, which are mainly composed of lipids and proteins. Based on this tissue structure an application of lipid-unionized compounds would be preferable for therapeutic treatment [58]. Furthermore, the penetration across epithelial borders is a slow process due to the effect of the barrier properties. The driving force in passive penetration between two compartments is based on the concentration differences across the separating membrane [8]. In addition, many drugs for local application do exist in their ionized form, thus rendering them ineffective for membrane permeation.

In ophthalmology, to obtain therapeutic ocular concentrations especially in the posterior segment one has to administer drugs systemically and thereby exposing the body to possible drug related general side effects. Other mode of drug application is by topical eye drop administration which is dependent on the above-mentioned passive drug tissue penetration properties.

The solution against the limitation of drug penetration due to tissue barriers and passive distribution could be in the application of an external electrical field. The basic principle of electricity, that like charges repell and opposite charges attract each other could be used for local ionized drug administration through tissue borders. Thus, in order to deliver a negatively charged drug, the negative electrode (cathode) is placed on epithelial surface where it is repelled and is attracted towards the positive (anode) one, which is placed elsewhere on the body. As for the administration of positively charged drugs the positive electrode is placed on the surface area and the opposite electrode is applied somewhere else.

1.1.3. Theory

Iontophoresis could be defined as an enforced penetration of charged molecules through a tissue border by applying an electrical field. Several authors tried to explain the different mechanisms of the ion transport during iontophoresis on skin. Abramson and Gorin derived an equation to correlate the iontophoretic flux to electric mobility, electroosmosis, and simple diffusion [59]. Other authors used a modified form of Nernest-Planck flux equation to explain the mechanisms of ion transport during iontophoresis application [60-61].

In summary, the increased penetration of an ionic compounds achieved by applying an electrical field can thus be due to the electrochemical potential gradient across the skin, increased skin permeability under the applied electric field, and a current – induced water transport effect (electroosmosis or convective transport) [62-63].

$$\mathbf{J^{isp} = J^{p} + J^{e} + J^{c}}$$

J^{p} is the flux due to passive delivery and is defined by:

$$J^p = K_S D_S dC/h_s$$

K_s - partition coefficent between donor solution and stratum corneum

D_S – diffusivity across the skin **dC/h_s –** concentration gradient across the skin

J^e is the flux due to electric current facilitation and is defined by:

$$J^e = [(Z_i D_i F)/ R T] C_i dE /h_s$$

C_i – donor concentration of the ionic species i

Z_i – electric valence of ionic species I **D_i** – diffusivity of ionic species in the skin

F – Faraday constant **T** – absolute Temperature

R - gas constant **dE /h_s –** lectric potential gradient across the Skin

J^c is the flux due to connective transport and is defined by:

$$J^c = k C_S I_d$$

k – proportionality constant **C_S** – concentration in the skin tissue

I_d – current density

In ophthalmology, it is due to the work of Hughes and Maurice who have detailed the factors influencing the iontophoresis as they formulated the equation demonstrating the drug quantity (m_d), which is penetrating the epithelium [30].

$$m_d = (i P_d C_d t)/ [F (P_d C_d + P_i C_i)]$$

i – current density (the surface area over which the current is applied)

t – duration of the iontophoresis **C_d** – drug concentration

C_i – concentration of competing ionized substances **P_d** – tissue permeability to the applied drug

P_i – tissue permeability to the competitive ions **F** - Faraday's constant

1.1.4. Factors affecting iontophoretic transport

Table 2. Factors affecting iontophoretic transport [64]

Factors influencing iontophoretic penetration
1. Drug concentration
2. Current density
3. Duration of iontophoresis
4. Skin impedance
5. Drug lipophilicity/hydrophilicity and molecular weight
6. Ion mobility/conductivity
7. Ionic valance
8. pH of the drug solution
9. State of ionisation
10. Effect of iontophoresis on drug metabolism and degradation in the skin

The major factors influencing the iontophoretic compound penetration can be divided into the physiochemical properties of the compound itself (molecular size, charge, concentration), the solution factor (type of the buffer, pH, presence of other compounds), the electrical and technical factor (different types of current, electrodes, treatment length, current density), biological or physiological variations (site, humidity, regional blood flow).

As already mentioned, iontophoresis is a procedure of ion movement enforced through the application of an electrical field. Thus the optimum pH for iontophoretic delivery is where the compound exists mainly in its ionized form. This was demonstrated for different solutes [65-66]. In several studies the permeability coefficients of a series of positively, negatively and uncharged solutes across excised human skin have been shown to be a function of the molecular size [67]. The hypothesis is that as the molecular size increases, the permeability coefficient does decrease [67-68]. Based on a free volume model [69] the molal volume of 150cm^3/mol [67] was considered optimal for iontophoretic penetration through skin. However it was successfully demonstrated that solutes with a relatively higher molecular weight, such as insulin, were penetrating effectively across the skin by using iontophoresis [62, 70-72].

Only a few studies examined the effect of ionic charge of drug molecules on iontophoretic drug delivery. It was observed that monovalent molecules could be delivered more readily than divalent ones, even if they had similar molecular weight. This suggests that divalent ions may interact more strongly with charged sites in the skin than monovalent do. Thereby resulting in a slower migration [73-74].

It was demonstrated that a rising concentration of the drug solution in the donor compartment resulted in an increased steady-state flux of drug solutes [75-79]. At higher concentrations it is possible that the transport becomes independent due to saturation of the boundary layer in relation to the donor solution [74].

The duration and the current strength play an important role in drug penetration by iontophoresis administration. Several studies demonstrated that a linear relationship between the flux of a number of compounds and the applied current exists [74-75, 77, 80-84].

Similar results in the ocular penetration of cefazolin, ticacillin and gentamicin confirm the correlation with the current strength [34, 54].

However the application of the current is limited by patient safety considerations such as pain and possible electrical tissue damage due to burns or cell necrosis.

It is known that by applying an electric field, all ions of a drug solution are affected [77, 80]. Ideally, the use of a buffer system should be avoided in iontophoresis, but if this is not possible, alternate buffers consisting of ions with low mobility or conductivity are preferred. It is preferable that the active charged drug ions should carry more than one-half of the conductivity of the solution [85].

1.1.5. Pathways

Different possible compound penetration mechanisms were studied and hypothesized for iontophoretic application on skin. The shunt pathway is believed to be major iontophoretic transport route via hair follicles [86-87] and sweat ducts and sebaceous glands [59, 88]. Further observations were made for artificial pores as a result of temporary disruption of the stratum corneum due to the electric current [89-90]. Another possible mechanism is the transfer by “flip-flop”. This theory based on the hypothesis that the skin permeability is changeable through the influence of electric current [64, 81, 91-93]. When an electric potential is applied across a cellular membrane a voltage-dependent “flip-flop” of polypeptides helices occurs. This causes a rearrangement of the helices to a parallel fashion. All partially negatively charged oxygen is then attracted to the positive site of the membrane leading to a repulsion between the neighboring dipoles within the structure of the polypeptides. In this manner, voltage –dependent pores are formed [59, 93-95]. Monteiro-Riviere et al. demonstrated by observation with an electronic microscope that the transdermal penetration of mercury took place via intra- and intercellular pathway [96]. Another mechanism is that of electroosmosis. It was shown that the transport of uncharged electrodes could be facilitated by either cathodal or anodal iontophoresis by the process of electroosmosis [97]. An electrically driven flow of ions across a membrane having a

net charge can induce a coupled flow of solvent. This effect is pH dependent. In physiological conditions the induced volume flow during iontophoresis is in the direction of positive ion transport supporting the hypothesis of cation selectivity of the skin [98-103].

In ophthalmology iontophoresis is applied either in a transcorneal or transscleral fashion. Until now the precise mechanisms of ocular drug penetration are not identified. The differences of the tissue structure in comparison to the skin do not allow a simple application of the mentioned iontophoretic pathways for the ocular tissue penetration.

1.1.6. Advantages

The application of iontophoresis as a non-invasive and local drug administration method has the advantage of a drug delivery, which bypasses the hepatic first pass effect and gastrointestinal vagaries. Furthermore in the case of systemic drug delivery through the skin by application of iontophoresis controlled plasma levels of potent drugs with short half-lives could be obtained and thereby facilitating the drug delivery and increasing patient compliance [63]. The peaks and troughs associated with injections can be accurately controlled and titrated over a period of time by employing iontophoresis [104]. The passive transdermal drug delivery is usually limited to the delivery of small, nonpolar, lipophilic solutes. Iontophoresis facilitates the transport of charged molecules and even of those with higher molecular weight. The inter-and intrasubject variability is considerably reduced in iontophoresis since the rate of drug delivery is proportional to the applied current [74, 77, 80-81].

The benefit of iontophoretic drug delivery in ophthalmology lay in its capacity to obtain high intraocular and especially posterior pole drug tissue concentration in a controlled and safe fashion, while minimizing the systemic drug exposure [29]. The ease of administration and the potential possibility to apply for example transscleral CCI (Coulomb-controlled iontophoresis) on a repetitive basis make this treatment modality of special interest in chronic and long-term intraocular diseases [51-53]. This non-invasive drug delivery system minimizes the risk of trauma due to drug delivery ways such as intravitreal or peribulbar injections, possible risk of infection and inflammation and hemorrhages. Its advantage in treating local conditions lies in the reduced incidence of systemic side effects due to minimal systemic uptake of the administered drugs and in high local drug concentrations [9, 105].

1.1.7. Risks and disadvantages

Like every drug administration method, the possibility of unwanted side effects exists also in the application of the iontophoresis, which can be due to the application itself or to the administered drug or the combination of both factors. Side effects such as erythema, skin irritation or minor burns were observed in iontophoretic applications in dermatology [63, 106]. In the past, ocular iontophoresis studies demonstrated that this application method was not without risks [29]. The safety of the iontophoresis depends mainly on the administered current density to the surface of the eye. For transcorneal iontophoresis a density of 20mA/cm^2 for a treatment duration of 5min appears to be without ocular risks [30]. In the case of transscleral iontophoresis, it seems that, if a current controlled iontophoresis is applied, a current density of 25mA/cm^2 permits an increased drug delivery without the occurrence of ocular lesions [107]. In previously published studies, high current densities were administered to a small ocular surface resulting in localized electrical burns [108], corneal epithelial or conjunctival edema, mucous discharge [37] decreased corneal endothelial cell counts [39]. After transscleral iontophoresis histopathological changes, such as hemorrhagic necrosis, edema, and infiltration of polymorphonuclear cells could be observed [29, 109].

Furthermore chemical tissue damages could be due to the pH of the applied drug solution, which might be acidic or basic, or due to changes in the pH occurring while the iontophoretic administration due to electrolysis.

Table 3. Lesions observed after transcorneal iontophoresis

author/year	**drug**	**current density [mA/cm]**	**duration [min]**	**probe diameter [mm]**	**animal**	**observed lesions**
Hill/1978 [44]	vidarabine	0.5	4	10.0-20.0	rabbit	epithelial
Hughes/1984 [30]	fluorescein	251	5	10.0-20.0	rabbit	stromal edema
Rootmann/1988 [37]	tobramycin	0.8	5	11.0	rabbit	epithelial
Choi/1988 [39]	vancomycin	7.1	5.0	3.0	rabbit	8.8 % loss of endothelial cells
Grossmann/1990 [48]	ketoconazole	21	15	3.0	rabbit	corneal opacity
Grossmann/1990 [48]	NaCl 0.09%	2.8	10	3.0	rabbit	endothelial cell loss

Table 4. Lesions observed after transscleral iontophoresis

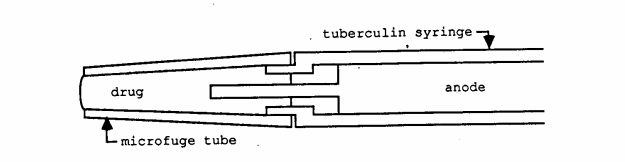
author/year	drug	current density [mA/cm^2]	duration [min]	probe diameter [mm]	animal	observed lesions
Yoshizimi/1997 [110]	foscarnet	530	10	0.19mm^2 probe tip surface	rabbit	Localized necrosis of the retina, choroids, pigmented epithelium
Barza/1986 [34]	gentamicin	255	5	1.0	rabbit	hemorrhagic necrosis, inflammatory infiltration of the retina, choroids, ciliary body
Barza/1986 [34]	cefazolin	127	10	1.0	rabbit	
Barza/1987 [35]	gentamicin	765.3	10	0.5	monkey	retinal necrosis
Lam/1991 [108]	NaCl 0.09%	531	15	0.7	rabbit	retinal necrosis

1.1.8. Units/Devices

A variety of different iontophoresis devices exists. For the application of iontophoresis in dermatology 3 types of devices are commercially available: mains-powered units, simple battery-poweredunits, and rechargeable power sources [108]. For example, the battery-operated device (Motion Control Inc. Salt lake City, UT) generates a precise dose of direct current irrespective of changes in skin resistance and has an automatic control for shut off if the skin/electrode resistance exceeds the pre-set limits [111]. The Hidrex device (Gesellschaft für Medizin und Technik, Wuppertal; Germany) can be operated by a rechargeable energy source or by batteries [112-113]. Furthermore it is designed for home use and characterized by safety equipment, automatic timing, and remote control for amperage adjustment. New devices using two-pulsed direct current (d.c.) have been described: Advanced Depolarizing Pulse Iontophoresis System (ADIS-4030) and Transdermal Periodic Iontotherapeutic System (TPIS). The TPIS is capable of delivering pulsed d.c with various combinations of waveform, frequency, on/off ratio, and current ration for a specific duration of the treatment [62]. The ADIS device is designed to give a continuous delivery under constant application of pulse current [114]. Furthermore, two different power supplies do exist a constant current power supply, which allows direct monitoring of the delivered current or constant voltage power units.

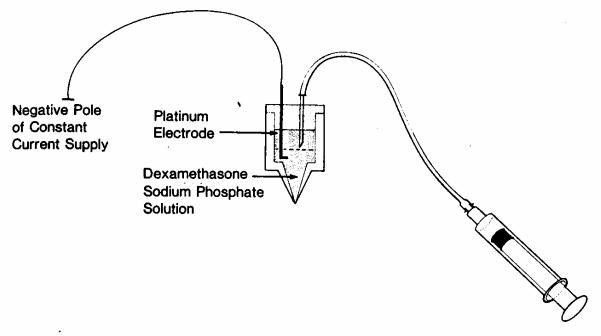
As already mentioned, the current density is the principal parameter of determining the rate of drug penetration through the different tissue barriers. In ophthalmology, the iontophoresis application resulted, therefore, in the design of several types of ocular electrodes, varying with the parameters such as shape, size and location area.

Figure 1.



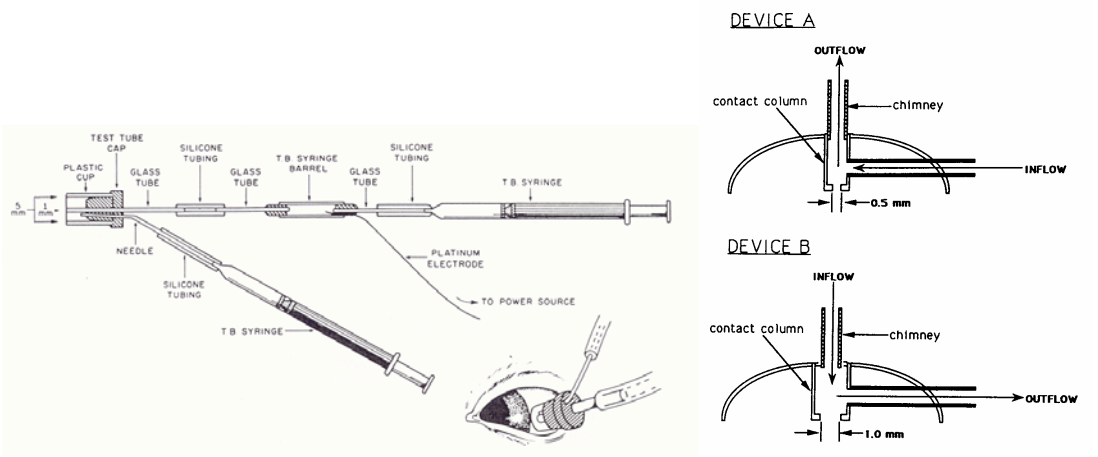
Schematic diagram of the ocular electrode used for transscleral and transcorneal iontophoresis by Choi, Lee and Grossmann [39].

Figure 2.



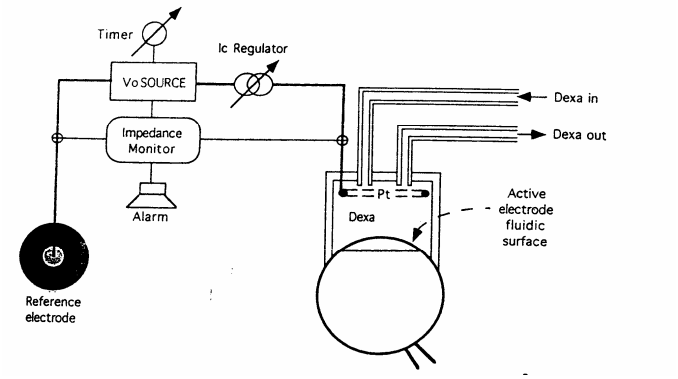
Design of the ocular application electrode used for transscleral iontophoresis by Lam et al. The pointed tip solution chamber was applied to the conjunctival surface of the rabbit eye [50].

Figure 3.



Ocular electrode used for transscleral iontophoresis by Barza et al [34].

Figure 4.



Transcorneal electrode used by Behar-Cohen et al [51].

1.2. Retinoblastoma

1.2.1. Cell and molecular biology of the retinoblastoma

Retinoblastoma is the most common intraocular malignancy in children. Human retinoblastoma occurs in two different forms: a nonheritable form, which is usually unilateral, and a heritable form, which is usually bilateral with autosomal dominant expression. In the case of the heritable form it can be associated with intracranial neoplasms and sarcomas later in life [116-117]. Knudson et al. hypothesized a “two-hit” mechanism for the development of retinoblastoma tumors [118]. This theory suggests that the development of retinoblastoma tumors would require two separate rate-limiting genetic events to occur. In the sporadic, unilateral form of retinoblastoma, both events would happen within a single retinal cell lineage, which is a rare occurrence. In contrast, patients with the heritable form of retinoblastoma carry within their germline a genetic defect either inherited from a parent or arising as a mutation in the germline. These patients would therefore have received the first “hit”, or genetic alteration, in the form of this mutant allele, and any retinal cell receiving a second hit would have malignant potential. Thus, if the frequency of second hits were sufficient, multicentric, bilateral tumors would be seen [119]. Initially Knudson and co-workers’ hypothesis was supported by statistical calculations but the discovery and cloning of the transgenic retinoblastoma RB1 gene, now regarded as the prototypical tumor suppressor gene, strengthened this theory profoundly. The RB1 was located on the long arm of chromosome 13 (13q14) by chromosomal studies in certain patients with bilateral retinoblastoma associated with cytogenetic abnormalities [120-121]. Finally the RB1 gene was identified and cloned [122-126]. The identification and cloning of the RB1 gene enabled genetic testing of normal cells and tumor cells in patients, which were diagnosed with retinoblastoma. Analysis of the RB1 allele in the blood cells of patients with bilateral or familial retinoblastoma generally revealed that a mutation is present in all cells, which functionally inactivates one copy of the RB1 gene. In the case of tumor cells from these patients, mutations in both RB1 alleles were observed, resulting in abnormal RB1 gene function. The second hit is therefore the loss of this second RB1 allele, presumably by spontaneous mutation [127]. Similar analysis of nonfamilial, unilateral cases did not demonstrate this germline mutation. However, tumors of both heritable and sporadic disease have mutations or deletions in both copies of the RB1 allele [128]. It remains unclear whether additional genetic alterations may also be required for the onset or progression of retinoblastoma. The RB1 gene consists of about 200 kilobases (kb) of DNA divided into 27 exons (RNA coding regions) separated by non-encoding segments, and is expressed ubiquitously [129]. A promotor region controlling transcription of the gene, is located at the 5’ (upstream) end of the gene. Genetic studies of tumors led to the identification of

two distinct families of genes: the proto-oncogenes promoting cell growth and the antioncogenes, or growth-suppressor genes, restricting cell proliferation. The RB1 gene, an antioncogene, encodes a protein of 105 kD, pRB, which functions as a central component of the cell cycle regulatory mechanism [130]. PRB exists in a hypophosphorylated (active) and hyperphosphorylated (inactive form). The normal pRB functions in the late G1 phase of the cell cycle by blocking the G1 progression, thus preventing the DNA synthesis and cell proliferation. The pRB in its hypophosphorylated form blocks the cell cycle by binding to transcription factors such as E2F [131-134], which are necessary to initiate the genes that control DNA synthesis during S phase [135-136]. As the cell traverses the restriction point and enters late G1, pRB becomes hyperphosphorylated and releases E2F, allowing the cell to enter into S phase [137]. The pRB remains phosphorylated throughout the remainder of the cell cycle until the completion of mitosis and re-entry into G1. The phosphorylation (inactivation) requires a group of enzyme complexes called the CDK4-6/ cyclin D kinases to regulate and catalyze this process [138-141].
Mutational inactivation of the RB1 gene leads to loss of pRB expression, thus allowing for constitutive E2F function and unregulated cell proliferation [142-143].
In summery, the RB1 protein product is found in most normal proliferating cells as it plays a key role in the cell cycle regulation. In addition to its crucial role in cell cycle control, pRB also appears to be essential for normal terminal differentiation of retinoblasts into mature retinal cells, and may be important in differentiation in many other tissues [144].
Most retinoblastomas appear to originate from retinal cells of immature neural epithelium (inner layer of the optic cup). These immature cells have the potential to differentiate into photoreceptors and Müller cells [145]. Studies of cell culture lines of human retinoblastoma have suggested more similarities to photoreceptor cells [146-149], showing cone-specific phenotypes such as transducin, cone photopigments (red and green opsin) and cone phosphodiesterase.
Histologicaly, two types of multicellular structures are common to human retinoblastoma. Small, hyperchromatic cells with large nuclei are seen in rosettes, which are most characteristic of human retinoblastoma (Flexner-Wintersteiner rosettes). These rosettes consist of a single layer of cuboidal cells surrounding a central lumen, into which project regular cytoplasmic processes resembling primitive photoreceptor differentiation. Similar single-layered rows of tumor cells surrounding a central lumen that is filled with neurofibrils (Homer-Wright rosettes) are frequently present in human retinoblastoma, but are also seen in neuroblastoma, medulloblastoma, and other primitive neurogenic tumors. The retinoblastoma tumor shows the tendency to be locally invasive into the choroid, vitreous, and optic nerve [119].

1.2.2. Diagnosis

Though being the most important intraocular malignant tumor in childhood it has a relatively rare incidence of about 1 in 15,000 live births in the United States [150]. The incidence of retinoblastoma among various populations is remarkably constant, providing strong evidence that the environmental influences are deniable in the etiology of this tumor [151]. In the United States nearly 90% of all cases of retinoblastoma were diagnosed before the age of 5 years [152]. However, some advanced-age manifestations of retinoblastoma diagnosis are reported in literature [153-156]. A hypothesis for the rare onset of retinoblastoma was proposed in the possible persistence of rare embryonal retinal cells [157].

In the United States more than 50% of all retinoblastomas where suspected or diagnosed after the observation of leukocoria. Strabismus, another common sign, is due to either the tumor or subretinal fluid associated with the tumor in the macula. The immediate cause of strabismus is a result of decreased visual acuity in an eye by either destruction or occlusion of the fovea.

Other less common signs and symptoms include a red, painful eye with glaucoma, poor vision, and an orbital cellulites-like condition [158]. However, it occurs to be unable to distinguish the observed lesions from simulating lesions. The most common causes of pseudoretinoblastoma were persistent hyperplastic primary vitreous, Coat’s disease and presumed ocular toxocariasis, congenital cataract and retinopathy of prematurity.

Therefore, children with intraocular lesions suggestive of retinoblastoma should be referred to ocular oncology centers for further evaluation. The workup of a child suspected of a retinoblastoma should consist of a family history checkup, dilated fundus examination, extensive physical examination by a pediatrician, an imaging study, and a staging examination under anesthesia. The most helpful diagnostic tools are CT, ultrasonography and MRI [159]. In new retinoblastoma if there are no other signs that the tumor has spread outside the globe routine bone marrow aspiration and biopsy, as well as lumbar puncture were considered not necessary [160]. In contrast, in cases in which there was clear evidence of tumor outside the eye, the full metastatic workup should be pursued.

It is of crucial importance to carry out a careful classification and staging examination under anesthesia before any further treatment. A detailed evaluation and exact documentation of every feature of the examination should be done, including the anterior segment, the iris and the vitreous cavity. Digital retinal pictures taken with the RetCam, wide-angle retinal photography and retinal drawings of both eyes are of tremendous importance. B-scan ultrasonography can be used to document the dimension and to demonstrate possible calcification within the tumor.

1.2.3. Classification of retinoblastoma

The most widely used staging system is the Reese-Ellsworth classification, which was developed in the 1950s for the prediction of the survivability of the eye after External Beam Radiotherapy (EBR).

Table 5. [160]

REESE- ELLSWORTH CLASSIFICATION
Group 1-Very favorable
a) Solitary tumor, less than 4 disc diameters (DD) in size at or behind the equator
b) Multiple tumors, none over 4 DD in size, all at or behind the equator
Group 2-Favorable
a) Solitary tumor 4 to 10 DD in size at or behind the equator
b) Multiple tumors, 4 to 10 DD in size, behind the equator
Group 3-Doubtful
a) Any lesion anterior to the equator
b) Solitary tumors larger than 10 DD behind the equator
Group 4-Unfavorable
a) Multiple tumors, some larger than 10 DD
b) Any lesion extending anteriorly to the ora serrata
Group 5-Very unfavorable
a) Massive tumors involving over half the retina
b) Vitreous seeding

Another classification system for the prediction of the globe salvage was developed in a major European retinoblastoma center in Essen, Germany [161]. Though more adapted to the new standards of ophthalmologic examinations and treatments, this classification has unfortunately not achieved a widespread usage.

Table 6. [160]

THE ESSEN CLASSIFICATION FOR CONSERVATIVE SIGHT-SAVING TREATMENT OF RETINOBLASTOMA
Group 1-Very favorable
• Tumor up to 4 DD and 4 diopters (D) elevation except tumors near the macula or adjacent to the disc
Group 2-Favorable
• Moderate size tumor(s) of 8 to 10 DD if not belonging in groups 3 or 5 for other reasons
• Tumor near the macula, even if small
Group 3-Doubtful
• Tumors adjacent to the disc, even if small
• Tumors with retinal detachment
• Moderately sized tumors with limited seeding over the surface
• Small, highly elevated tumor and therefore not separable from the ora serrata
• Tumors in this group only if mot belonging into groups 4 and 5

Group 4-Unfavorable
• Extensive tumor growth with or without limited seeding or retinal detachment • Large tumors adjacent to or overlapping the disc • Large tumors not separable from the ora serrata by ophthalmoscopy
Group 5-Very unfavorable
• Massive tumor growth up to half of the retina with or without diffuse vitreous seeding • Totally detached retina

A new classification system based on possible eye salvage with chemotherapy application as primary treatment of the intraocular malignancy has been proposed.

Table 7. [159]

NEWLY PROPOSED CLASSIFICATION FOR INTRAOCULAR RETINOBLASTOMA
Group A
• **Advantageous** location and size disease. **Avoids** significant morbidity and vision loss. One or more intraocular tumors 3 mm or less in greatest diameter; none touching the optic nerve or impinging on the foveal avascular zone. **No** vitreous seeding or subretinal fluid.
Group B
• **Brachytherapy**-eligible disease. Solitary retinoblastoma outside zone I with a basal diameter no larger than 10 mm **OR** multiple, closely spaced, smaller tumors confined to a single retinal area no greater than 10mm in diameter. **No** diffuse vitreous seeding * or significant † retinal detachment (SDR).
Group C
• Confined disease of a size requiring **chemotherapy**. one or more intraretinal or endophytic tumors, none exceeding 15 mm in greatest basal diameter. **No** local or diffuse vitreous seeding or significant † retinal detachment. Small tumors (<3 mm) touching the optic nerve or involving the fovea
Group D
• **Dispersed, disseminated**, or **diffuse** intraocular disease. Vitreous seeding or significant †retinal **detachmen**t, or both, may be present. The total volume of the tumor does not exceed half of the volume of the eye. **No** detectable extraretinal disease except for vitreous involvement. Potential for useful vision.
Group E
• **Extraretinal‡** retinoblastoma or the presence of intraocular tumor volume greater than half the volume of the eye. Primary **enucleation** recommended. Anterior segment disease, glaucoma, hyphema, total detachment with fixed retinal folds.
Group F
• **Future** risk from containment failure. Neuroimaging or histologic evidence of increased risk for metastatic disease (massive choroidal involvement and/or tumor in the optic nerve posterior to the lamina cribrosa).

* Localized vitreous seeding is allowed no greater than 2 mm from the surface of the tumor. † Significant retinal detachment (SRD) is defined as an area of detachment equal to or greater than the retinal area occupied by the tumor. ‡ Extraretinal retinoblastoma defines the disease extending beyond the retina and the vitreous.

1.2.4. Management of retinoblastoma

Enucleation, which was introduced more than 100 years ago, is still a major treatment indicated in cases with no hope of vision salvage or if invasion of the massive tumor into the optic nerve, choroid or orbit is present [161]. The most important consideration is to obtain a sufficiently long piece of the optic nerve for avoiding further extraocular spread of the tumor [163]. Nevertheless, a substantial decrease in the frequency of enucleation in the case of unilateral and bilateral retinoblastoma was observable in recent decades [164].

Retinoblastoma is generally a radiosensitive tumor. External beam radiation has been employed in the treatment of retinoblastoma since 1903, though modern treatment modalities utilizing megavoltage radiation were not introduced until the early 1970s. This treatment modality is a method of delivering whole eye irradiation to treat cases of advanced retinoblastoma, in particular those with diffuse vitreous seeding. External beam radiotherapy offers the potential of sight preservation, but has significant, real, and potential limitations. First, late-onset radiation complications, including radiation retinopathy, radiation vasculopathy, radiation optic neuropathy, cataract, and neovascular glaucoma may occur in as many as forty percent of treated cases. Second, the heritable component of retinoblastoma appears to generate a cancer diathesis, with up to 35 percent of children with retinoblastoma developing a second malignancy, often as late as three to five decades after primary treatment [165].

Recently, the management of retinoblastoma has evolved away from radical aggressive treatments such as enucleation and external beam radiation, towards more focal, conservative treatments or moderate, combined treatment modalities. Due to earlier detection, better patient and family monitoring, and advances in the therapeutic armamentarium of oncology, more specific treatment techniques for the different stages of the tumor have improved the prognosis for vision and life significantly for the most common intraocular malignant tumor of children. Although a variety of treatment modalities exist including enucleation, external beam radiotherapy, scleral plaque radiotherapy, laser ablation, cryoablation, laser hyperthermia, and chemotherapy, the optimal conditions for complete tumor control and minimal side effects are still under investigation [150, 161, 166-167]. The suitability of each technique is governed by factors such as tumor size and extent, vitreous seeding, site of involvement, patients' systemic status, and furthermore, by the inherent side effects of the treatment [168]. Plaque radiotherapy is a method where a radioactive implant is placed on the sclera over the base of the tumor and thus irradiating it through the sclera. In general, this treatment is limited to tumors less than 16 mm in base and 8 mm in thickness. Brachytheraphy can be used as primary or secondary treatment [169]. A 90% cure rate was reported in patients in the Reese-Ellsworth groups 1 through 3 [170].

Two different concepts for laser management in retinoblastoma exist: photocoagulation and hyperthermia. Laser photocoagulation is a method of treatment for small posterior retinoblastomas with different kind of lasers such as argon, diode or xenon laser. The size of the tumor is crucial for the success of this treatment, which is limited to tumors of 4.5 mm or less in base and 2.5 mm or less in thickness with no evidence of vitreous seeding [171-172]. Thermotherapy is a treatment where heat is delivered to the eye globe using microwaves, ultrasound or infrared radiation. When applied alone, the goal is to heat the tumor to 45 to 60ºC, resulting in a gray-white scar at the treated site. The heat can be delivered to the whole eye, sparing the anterior segment or focused on one specific localization [173].

Cryotherapy is usually applied in tumors anterior to the equator and in peripheral small retinoblastomas.

The treatment is promising if limited to tumors measuring 3.5 mm or less in diameter and 2.0 mm or less in thickness, with no evidence of vitreous seeds [174].

The most significant advance in the management of retinoblastoma occurred in the application of chemotherapy. To improve the rates of ocular salvage, systemic chemotherapy has become a mainstay in the initial management of retinoblastoma (Reese-Ellsworth stage III-V) [175-177]. This change was a result of new drugs such as carboplatin, VP-16 (etopside), VM-26 (teniposide), and cyclosporin, which all demonstrate an increased ocular penetration. This enables the use of more focused and selective treatment with combination modalities with remarkable results [178-182]. Chemotherapy can be used as a neoadjuvant treatment (chemoreduction), to induce a reduction of the tumor size followed by focal treatments. The combination of chemotherapy and heat is termed thermochemotherapy (TCT).

Administered as adjuvant treatment, chemotherapy is given to prevent metastasis in patients with increased risk of extraocular tumor spread.

The prognosis for the life and the vision of patients diagnosed with retinoblastoma has improved tremendously during the last century. But huge differences still exist in the survival rate between western and developing countries do exist. This difference in the survival rate is due to a delay in receiving medical attention and the standard of medical treatment possibilities offered for an adequate and effective treatment. Nevertheless remarkable results were achieved. One hundred years ago a diagnosis of retinoblastoma had nearly always fatal consequences. By introducing new treatment modalities the prognosis was gradually improving. For example only approximately 30% of the affected patients survived in the 1930s. In contrast, already 80% of the diagnosed patients survived in the 1960s. Finally the survival rate reached a level of nearly 95% in the 1990s [150, 166, 183].