SOME MORPHOPATHOLOGICAL ASPECTS OF THE EXPERIMENTAL EYE INFECTION WITH HERPES SIMPLEX VIRUS TYPE 1 IN RABBITS, FOLLOWED BY A TREATMENT WITH AQUEOUS FLAVONOIDS SOLUTION OBTAINED FROM PROPOLIS

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Introduction

The successes of the antiviral therapy, described by several authors (3, 9, 12, 15, 16, 18), are subject to a certain criticism, especially as regards the difficulty of applying the respective medication in the clinic, its toxicity and the way of administration (11, 13, 17).

The antiviral drug, be it either of a natural origin, or the result of a chemical synthesis, must act on the virus, either by blocking its penetration in the host cell, or by blocking the replication and the spreading of the virions, as well as by its capacity to stimulate the recovery of the affected tissues from the morphofunctional point of view.

The production of an antiviral drugs regards, among others, the physical and chemical characteristics of the nucleic acids in the viral and host cell and the characteristics of the enzymes which codify them, in order to inhibit the virus synthesis process by the modification of the genetic information. The knowledge of these data is the base of the action and of the efficiency of the therapeutical agent.

The drug must fulfil the following conditions, on which depends its efficiency: solubility; stability, in order to have efficiency in time; it must not cause any reaction of the organism, thus preventing the intoxication; it must not have an immunity suppressive action; it must not be teratogenic, mutagenic and oncogenic; and it must be economical.

In this context, the flavonoids fulfil the conditions mentioned above and the aqueous extract facilitates the possibilities of an efficient administration on the mucous membrane.

The capacity of certain components, the flavonoids included, to act on the enveloped viruses (1, 3, 5, 15, 19) group, which includes the Herpes simplex virus type 1, is well-known. The data in the specialized literature seem poor as regards the therapeutical utilization of the flavonoids in the eye infections with herpes virus, although these infections have different evolutions and morphoclinical aspects (14) and are quite frequent in clinic (6, 7, 10). Thus, it is estimated that over 90% of the children aged between 6 months and 3 years are infected with herpes simplex virus type 1 (12).

All these facts have determined us to use the flavonoids aqueous solution as eye drops in the experimental acute eye infections with Herpes simplex virus type 1 in rabbits, in order to observe its action and its therapeutical efficiency.

Material and Methods

In conventional Chinchilla rabbits, males and females, which weighed 2-2.5 kg and which were clinically healthy, after the sacrification of the cornea, we instilled 0.05 ml culture liquid with Herpes simplex virus type 1, in the conjunctival sack. The instillation was effected for both eyes with the same number of infective units. Each cornea was regarded as a separate reactive.

We used the following virus dilutions: 10^{-4}, 10^{-3}, 10^{-2}, with an infective title of $10^{-6.2}$ DIMCCT/0.1 ml, determined on primary cultures of total human embryo. This title was determined in paralel, in vitro.

The animals used in the experiment were divided in groups of two, from which three groups represented the control ones, namely, they were administered the above mentioned virus dilution. In other three groups, the experimental ones – after the instillation of the same virus dilution – we applied the treatment, by administering 0.1 ml of 15% flavonoids solution, with a 6.8 pH, obtained from propolis by means of ebbing, in the conjunctival sack.

The treatment started three hours after the administration of the virus, by the instillation of the same quantity, five times a day, every three hours. 30 minutes after the last inoculation of the solution, we applied a neutral ointment, in which we inbedded the aqueous flavonoids solution, in the conjunctival sack. This was followed by a 12 hours' break, then we applied the treatment again, by means of instillation of the aqueous solution. This treatment was applied for 7 days, without interruption, and by strictly observing the times of administration. Afterwards, there were 4 more days of treatment, by means of instillation of flavonoids solutions only, in the conjunctival sack every 12 hours.

All along the experiment, the animals were clinically followed. On the 4^{th} and the 7^{th} day since the inoculation of the virus, an ocular examination was effected, in order to observe the modifications which might have occurred.

On the 11th day since the beginning of the experiment, the animals were killed by means of exsanguinations and fragments of cornea were preserved in 10% formaline, then inbedded in paraffine, sectioned at 5 microns and coloured with hematoxyline-ecosine (H.E.) (by the Mann and Giemsa methods).

Results and Discussion

The general clinical examination of the animals pointed out a state of prostration and of listessness of the control groups, as well as of the treated groups. On the 4th day since the inoculation of the virus, in all the groups that were subject to our research, we noticed photophobia and a mucopurulent secretion at the nasal angle of the eye. On the 11th day of observation, the photophobia was still present in all the groups, but the ocular secretion had decreased in the case of the treated groups.

The histopathological examination pointed out the following:

In the control group in which we inoculated the virus in a 10^{-4} dilution, in certain areas of the corneal anterior epithelium, the Bowmann membrane was vaguely emphasized and the cells of the generating layer were swollen, with large, globular nuclei. The cells of the middle layer were large, with well outlined nuclei.

Under these areas, on large portions, a nodular infiltration, intensely populated with lymphocytes, histocytes, macrophages and, more seldom, with neutrophile granulocytes and erythrocytes, was present (Fig. 1). In the vicinity of the infiltration, towards the depth of the cornea, there were numerous newly formed blood vessels, with a relatively large lumen, with thickened walls, and swollen endothelial cells, which had large, vesicular nuclei, which proeminated in the lumen. There areas in which the anterior corneal epithelium was denuded. Under these areas, in the lamellate layer, on vast areas, an extended infiltration, of nodular structure, with the same cytological elements and newly formed blood vessels, was present.

In other areas, the anterior epithelium was almost intact. Nearby, in the corneal lamellate area, there was a lympho-histocytic and macrophagic diffuse infiltration, with a tendency to from groups of 5-8 cells. Near it, we noticed numerous newly formed blood vessels which lumen had various sizes.

Here and there, the anterior epithelium was thickened, on limited areas, which penetrated the cornea like some “spurs”. The thickening is the consequence of the increase in the number of cells in the generative layer and especially of the increase in the number of cells in the intermediate layer. Under these areas, we noticed infiltrating nodules, made of the same cytoelements and newly formed blood vessels.

By means of the Mann coloration, we pointed out numerous elements with an eosinophilic cytoplasm, with nuclei which chromation looked granulous, among the cells which constituted the infiltration. These cells with an eosinophilic or, in some cases, basophilic tinction, contained oxyphilic, oval or round granules in their cytoplasm.

In the control group, in which we applied the treatment after the administration of the virus in a 10^{-4} dilution, the basal membrane of the anterior corneal epithelium was very difficult to be detected. The cells of the basal layer were big. Beyond the epithelium, we could notice a slight oedema and a diffuse infiltration, with very few cells, mainly lymphocytes and histiocytes, with many pycnotic nuclei and new blood vessels, with very thin walls, a collapsed lumen and without any perivascular cellular infiltration, (Fig. 2). By using the Mann coloration, we did not notice any cells with particular tinctorial affinities of the cytoplasm.

In the control group in which we inoculated virus in a al 10^{-3} dilution, the cornea had denuded areas in the anterior epithelium. In other areas, the epithelium looked thickened, with numerous cellular ”spurs”, which penetrated the stroma of the cornea. In the vicinity of these areas, we noticed an extended nodular infiltration, with the same cell types and newly formed blood vessels (Fig. 3) as those of the previous control group.

In the experimental group in which the treatment was applied after the administration of the virus in a 10^{-3} dilution, the basal membrane of the anterior corneal epithelium was intact. The epithelial “spurs”, a consequence of the cellular hyperthropy of the basal layer, were very rare. The longitudinal and the cross diameter of the basal cells, did not have any significant differences for comparison. The subepithelial infiltration was made of small nodules, which were made of lymphocytes, histocytes, macrophages (Fig. 4) and of a few cells with an eosinophilic cytoplasm.

In the case of the control group in which we administered virus in a 10^{-2} dilution, the basal membrane of the anterior corneal epithelium was very vaguely perceptible. In certain areas, the epithelium was not modified. In other areas, the basal epithelial layer was made up of large cells, with a longitudinal diameter which was double the size of the cross diameter. This aspect created the impression of thickening of the tissue, but without increasing the number of layers of cells and without increasing the number or the size of the epithelial “spurs”. The nuclei of these cells had little chromatin. In the subepithelial area, we noticed slight a nodular infiltration, with the same cell type and with newly formed blood vessels, with thin walls and narrow lumen (fig. 5).

After the administration of the virus in a 10^{-2} dilution, followed by the treatment, the basal membrane of the anterior corneal epithelium was slightly visible. In certain areas, the epithelium was thickened and deeply penetrated the corneal tissue, as cellular “spurs”, with a rounded aspect. The basal cells were big, their longitudinal diameter being double in size in comparison to the cross diameter. In the subepithelial area,

we noticed an oedema and a diffuse lympho-histio-macrophagic infiltration, with rare cells and with newly formed blood vessels with thin walls and narrow lumen (Fig. 6). In the depth of the corneal lamellate layer, the diameter of the blood vessels was larger, but had a collapsed lumen. In the perivascular area, the cellular infiltration was poor. In the areas which had no blood vessels, the cytoinfiltration was more abundant and had no tendency to nodular organization. The cells with eosinophilic cytoplasm are rare. In the anterior corneal epithelium, we noticed rare specific inclusions, in all the control groups. They were not present in the treated groups. The inclusions were: intranuclear, oval, oxyphilic, very pale and had, in the middle, a slightly basophilic granule.

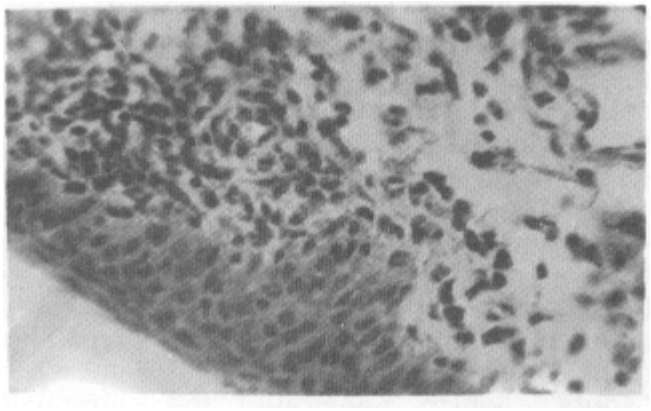
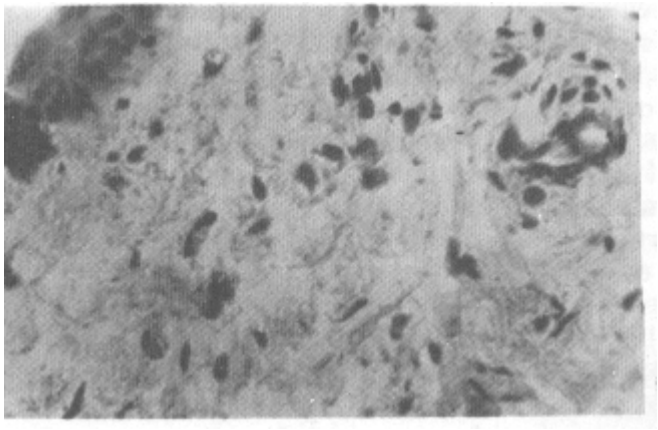


Fig. 1 – Virus administration in 10^{-4} dilution, control group
Cornea: Anterior epithelium with hypertrophic cells of the basal and parabasal layers.
In the subepithelial area, intensely populated lympho-histio-macrophagic nodular infiltration and newly formed blood vessels with thickened walls. Col. H.E. x 400

Fig. 2 – Virus administration in 10^{-4} dilution, followed by treatment
Cornea: Anterior epithelium with hypertrophied cells of the basal layer. In the subepithelial area, a slight oedema and a diffuse and rarely populated lympho-histio-macrophagic infiltration with pycnotic nuclei and newly formed blood vessels with collapsed lumen. Col. H.E. x 400



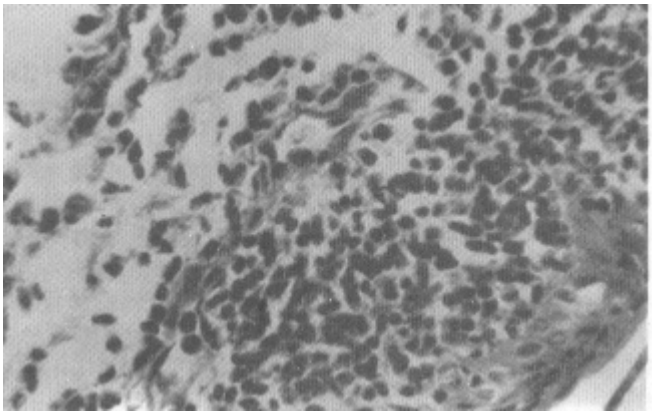


Fig. 3 – Virus administration in a 10^{-3} dilution, control group
Cornea: Anterior epithelium with numerous cellular “spurs”.
In the subepithelial area, nodular lympho-histio-macrophagic infiltration, on large areas, and newly formed blood vessels with large lumen.
Col. H.E. x 400

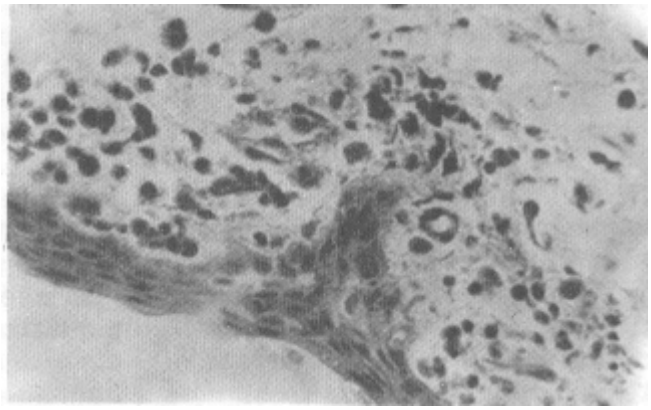


Fig. 4 – Virus administration in 10^{-3} dilution, followed by treatment
Cornea: Anterior epithelium with rare cellular "spurs".
In the subepithelial area, nodular lympho-histio-macrophagic infiltration, on reduced areas. Col. H.E. x 400

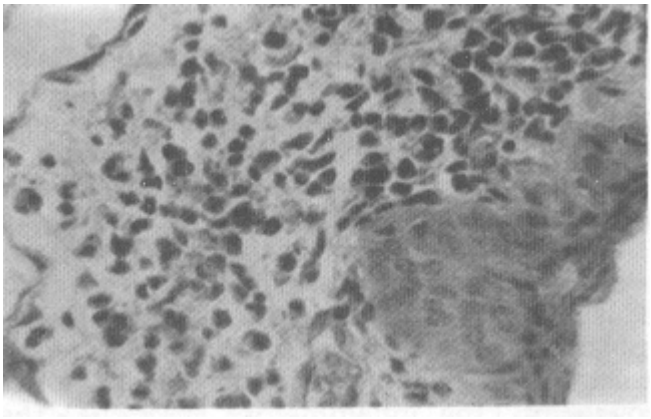


Fig. 5 – Virus administration in 10^{-2} dilution, control group
Cornea: Anterior epithelium with hypertrophied basal cells.
In the subepithelial area, medium populated nodular lympho-histio-macrophagic infiltration and, in its vicinity, newly formed blood vessels. Col. H.E. x 400

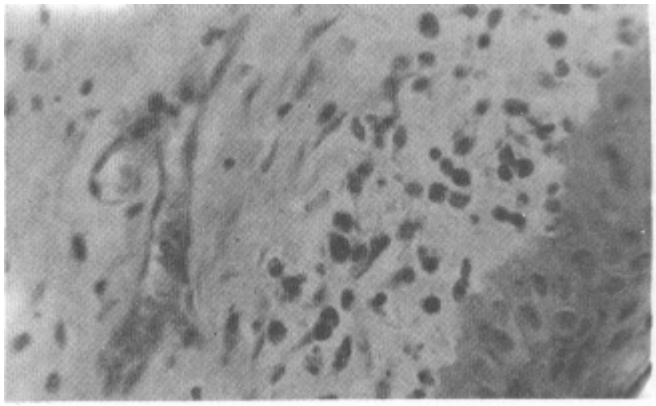


Fig. 6 – Virus administration in 10^{-2} dilution, followed by treatment
Cornea: Anterior epithelium with small cellular "spurs" with a rounded top.
In the subepithelial area, diffuse, rarely populated lympho-histio-macrophagic infiltration and newly formed blood vessels with thin walls and narrow lumen. Col. H.E. x 400

PORTOCALA (quoted by 11) presents the genesis, the dynamics, and the variability in size and in tinctorial affinity of the inclusions, which he considers to have various causes, among which the most important are: the virus strain and the way of administration of the virus. In this respect, in the case of the experiment above, we estimate that the appearance, the organization, the frequency, the coloration and the proportion of inclusions to the chromatic mass probably depended on the antigenic characteristics of the virus strain, and that their absence was determined by the quality and the effect of the therapuetical agent.

In comparison to the control groups, in all the treated subjects, the basal membrane of the anterior epithelium reappeared. This fact points out to the tendency of limitation of the pathogenic process and of

cytoarchitectonical recovery of the tissue, as it is well-known that, in the case of the unaffected eye, it is well defined from the morpho-functional point of view. In the healing process, we must point out the particular cytological dynamics – from hypertrophy to normotrophy – of the elements of the basal and the middle layers and the reduction in volume and the rounding of the epithelial "spurs". The epithelial karyokinetic index is low, fact which suggests a non-mitotic proliferation, as other authors have noticed as well. (11). The oedema of the corneal lamellate layer, the new vascularization and the presence of the diffuse or nodular lympho-histio-macrophagocytic perivascular infiltration, as well as of other cells, which may be attributed to the modification of the antigenic characteristics of the surface membrane of the stromal cells (2, 4, 8, 13, 14).

We may estimate the therapeutical effects by observing the epithelization of the cornea and the reduction of the inflammatory reaction. The epithelial and the stromal recovery was high in the case of the treated groups, in comparison to the control groups. The reduction of the inflammatory cytoinfiltration, the decrease and the collapse of the lumen of the newly formed blood vessels seem to be responses to the therapeutical factor. The morphological picture is different in the case of the control groups, in comparison to the one of the treated groups and represents the reaction of the host to the virus. The virus strains own large amounts of antigenic products which induce the inflammatory reactions. The pathomorphological picture may be determined by the variability of the viral genome (8, 19), as a consequence of the breakage and of the recombination of its DNA. Thus, glycoproteins with particular defects may be produced. They may be deposited sometimes and may affect the cornea in different manners. Therefore, the faulty glycoprotein is separated from the gene which determines the epithelial affectation, while each gene that produces the stromal lesion may be separated from other genes. Under these conditions, it may also be suggested that, after the direct invasion of the endothelium by the herpes virus, the result was a pleiomorphic aspect and a modified density of these cells.

The stromal affectation may also be a reaction of the host to the particular viral products, as we have already pointed out, and certain virus strains produce a certain quality and quantity of antigens which stimulate the evolution of a certain histopathological picture. If the glycoproteinic substances cause certain types of affectations, then, probably, the therapeutical agent, the flavonoids, will be able to inhibit the synthesis of the particular viral components which are responsible for the appearance of the lesions. In this context, the therapeutical agent stands out as an antiinflammatory substance, it decreases the cytoinfiltration in number and in diffusibility, it facilitates the resorption of the oedema, it reduces the number of newly formed blood vessels and controls the endothelitis.

Conclusions

1. In rabbits groups, after the scarification of the cornea, we administered 0.05 ml culture fluid with Herpes simplex virus type 1, in dilutions of 10^{-4}, 10^{-3} and 10^{-2} in the conjunctival sack. In some of the groups, namely in the experimental groups, we instilled 0.1 ml of 15% aqueous flavonoids solution, with a 6.8 pH, in the conjunctival sack, as a treatment. This solution was obtained from propolis by means of ebbing, by an original procedure, and its therapeutical effect was evaluated by means of histopathological examinations.

2. In the control groups, the anterior corneal epithelium had denuded or hypertrophic areas, forming cellular "spurs", which penetrated the layer beneath, while the nasal membrane was barely visible. Under the epithelium, an oedema and a diffuse and/or nodular lympho-histio-macrophagocytic infiltration were present, while in the vicinity, there were newly formed blood vessels, which were usually surrounded by the same type of cells.

3. In the treated groups, we noticed the tendency of epithelial and stromal recovery, by means of epithelization, while in the subepithelial area, there was a reduction of the oedema, of the cytoinfiltration and of the newly formed blood vessels and a histopathological appearance of recovery, which was significant in comparison to the control group.

4. The flavonoids aqueous solution, made of propolis, seems to inhibit the synthesis of the viral elements, responsible for the lesional picture, and stands out as an anti-inflammatory and epithelizing subtance.

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