CERTAIN MORPHO-PATHOLOGICAL ASPECTS RESULTING FROM THE EXPERIMENTAL INOCULATION OF THE PARAINFLUENZA VIRUS NO. 3 AND OF AQUEOUS FLAVONOIDS EXTRACT OBTAINED FROM PROPOLIS FOR THERAPEUTICAL PURPOSES TO MICE

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Summary

After the intranasal inoculation of the parainfluenza virus no. 3, strain no. 739, 2D, the therapeutical effect of an aqueous flavonoids extract obtained from propolis, by refluxing, by means of an original method and administered in an identical manner was observed.

The studies were effected on lots of standard homogenous mice, males and females which were clinically healthy. The animals were divided into control groups, for the virus and for the therapeutical agent, and experimental groups, in which the administration of the virus and of the therapeutical agent had an alternative priority. Three days after the last inoculation, the animals were killed, by exsanguinations, then they were necropsized and samples of their lungs were extracted for the general histopathological examination; for the histochemical examination for the acid mucopolysaccharides and for the total lipids; and for the histoenzymological examination for the succinic-dehydrogenase and the lactic-dehydrogenase, by using the adequate techniques.

From the other organs: brain, cerebellum, thyroid, thymus, trachea, heart, liver, pancreas, spleen, lymphatic gangliones, kidneys, surrenal gland, ovary, testicle, fragments were extracted and processed in order to effect the general histopathological examination.

After the application of the treatment, a particular morpho-pathological image was observed. Thus, in the experimental groups, the wounds were predominantly situated in the interparietoalveolar space, consisting of a slight hyperemia, oedema, hialinosis of the mean of the blood vessels and lympho-histio-macrophagocitary infliltration, which rarely populates the area. This aspect suggests the reduction of the pathological process and a tendency to recovery. The histochemical and histoenzymological image completes this picture and seems to draw our attention upon the restitutional phenomena. In other organs, for example the liver, a vacuolary distrophy of the haepathocytes may be noticed, while in the spleen, the presence of the megakariocytes may be observed and in the trachea, we may see denudation of the epithelial cilli.

The therapeutical agent, the aqueous flavonoids extract, administered nasally, was well tolerated by the organism. The effectiveness of the therapy seems obvious, particularly when the therapeutical agent is administered after the inoculation of the virus.

***Key words**: infection with parainfluenza virus no. 3, treatment, aqueous flavonoids extract, histopathological examination.*

The preparation of an antiviral drug, which is efficient from the pharmaco-dynamic point of view, has as an objective the possibility of an inhibition of the viral synthesis processes, or of troubling the genetic information, consequence of a nucleic acid with an abnormal structure.

The differences between the physical and the chemical characteristics of the nucleic, the cellular and the viral acids (McINTOSH, 1990), as well as the differences between the characteristics of the viral enzymes and those of the homologous cellular ones, which have as an objective exactly the aims previously mentioned (ESANU, 1989; ESANU, 1990; LANDOLFI et al., 1984; PERRIN et al., 1984), are well known. Therefore, we must take into consideration all these principles when a therapeutical product is elaborated. One must also consider the specificity and the lack of toxicity (ESANU, 1989; GALASSO et al., 1984; PERRIN et al., 1984).

Certain products, the flavonoids included, seem to represent a certain group that has a virucide activity. Thus, we know that the flavones derived from the 4'5' dihydroxy-3', 3', 7, trimethoxyflavone inhibit certain enteroviruses, such as the coxsachevirus, the poliovirus, and the rhinovirus *in vitro.*

These compounds have the capacity to inhibit the formation of the viral ARN-polimerase (GALASSO et al., 1984). We must mention the fact that these flavonoids have a similar action on the envelope viruses, as well as on the influenza, parainfluenza and herpetic viruses (GALASSO et al., 1984; HODNIC et al., 1988; PETICA et al., 1994).

As for the treatment trials in the infections with the parainfluenza virus no. 3, of which we must mention the utilization of the racomic epinephrine, of the corticoids and of dexamethisan, the results obtained seem uncertain; and the efficiency of the ribavirin manifests itself more *in vitro* (CHANOCK et al., 1990; HIRSCH et al., 1990).

Taking into consideration the virucide and antiinflammatory effect of flavonoids, pointed out by several authors (ESANU, 1989; ESANU, 1990; GALASSO et al., 1984; HODNIC et al., 1988; LANDOLFI et al., 1984; PETICA et al., 1994), in the present report, we have the objective of observing these effects, in an acute experiment, in mice, after the experimental intranasal inoculation of the parainfluenza no. 3 virus, followed by the administration, - on the same way – of the aqueous flavonoids extract obtained from propolis, for therapeutical purposes.

Material and Method

We used the parainfluenza virus no. 3, strain no. 739, 2D, with 1 DICT 50 x 10/0.2 ml infecting titre.

We used white, conventional mice, of both sexes, weighing 14-18 g, divided in groups of six, for the control, as well as for the experimental groups.

The therapeutical agent we used was the aqueous flavonoids extract, administered intranasally, under the form of solution, in a dose of 0.1 ml, in a concentration of 15% of polyphenols of the flavonoid type, with a pH of 6.8, obtained from propolis by refluxation by means of an original method.

The control groups

- group “A” includes the animals in which phosphate salt buffer was inoculated intranasally. This is the control group for animals and it was sacrificed 3 days after the inoculation.
- group “B” was inoculated the parainfluenza no. 3 virus (P13) and sacrificed 3 days after inoculation;
- group “C” to which only one dose (T1) of the therapeutical product was administered and which was sacrificed 3 days after this administration;
- group “D” to which two doses of the therapeutical product, at an interval of 72 hours, T1 + T2 (72 h), were administered and which was sacrificed 3 days after the last administration.

The experimental groups

- group “E” to which the parainfluenza no. 3 virus was inoculated and, after 3 hours, the therapeutical product was administered, Pl 3 + T1 (3 h), and which was sacrificed 3 days after the last administration;
- group “F” to which the therapeutical product was administered and, after 3 hours, the parainfluenza virus no. 3 was inoculated, T12 + Pl 3 (3 h), and which was sacrificed 3 days after the last administration;
- group “G” to which the parainfluenza no. 3 virus was inoculated and, after 24 hours, the therapeutical product was administered, Pl 3 + T1 (24 h), and which was sacrificed 3 days after the last administration;
- group “H” to which the therapeutical product was administered and, after 24 hours, the parainfluenza virus no. 3 was inoculated, T1 + Pl 3 (24 h), and which was sacrificed 3 days after the last administration;
- group “I’ to which the parainfluenza no. 3 virus was inoculated and, after 24 hours, the first dose of the therapeutical product was administered, followed – after another 24 hours – by the administration of the second dose of the same product, Pl 3 + T1 (24 h) + T2 (48 h), and which was sacrificed 3 days after the last administration;
- group “J” to which the first dose of the therapeutical product was administered and, after 24 hours, the parainfluenza virus no. 3 was inoculated, followed – after another 24 hours – by the administration of the second dose of the same product, T1 + Pl 3 (24 h) + T2 (48 h), and which was sacrificed 3 days after the last administration;
- group “K” to which the first dose of the therapeutical product was administered, then, after 24 hours, the second dose of the same product was administered, followed – after another 24 hours – by the inoculation of the parainfluenza virus no. 3, T1 + T2 (24 h) + Pl 3 (48 h), and which was sacrificed 3 days after the last administration.

At the end of the experiment, the animals were sacrificed by means of exsanguinations, then they were necropsized. Fragments of organs were taken from them and processed as follows.

The lung fragments, taken immediately after the animals were killed, were frozen and sectioned in the criotome. The indirect imunofluorescence and histoenzymological reactions were effected on the sections obtained, for the succinic-dehydrogenase (SDH), by means of the Nachlas metod; and for the lacticdehydrogenase (LDH), by means of the Hesse-Scarpelli-Pearse method. Other lung samples were fixed in 10% formol, a part of them being included in paraffin, sectioned at 5 microns, and coloured with haemathoxilin-eosine (HE). Van Giesen and Giemsa for general orientation, and with Alcian blue for acid mucopolysaccharides (AMPS), while the other part were sectioned in the criotome and coloured with Scherlach for total lipids (TL).

The fragments from the other organs – brain, cerebellum, thyroid, thymus, trachea, heart, liver, pancreas, spleen, mediastinal lymphoganglions, kidneys, surrenal gland, testicle, ovary, were fixed in 10% formol, included in paraffin, sectioned at 5 microns and coloured in HE, Van Giesen and Giemsa.

The results of the observations have been systemized by means of the descriptive presentation of the wounds and their incidence was statistically estimated. The intensity of the histochemical and histoenzymological reactions was noted with (+) weakly positive and (++) = obviously positive.

Results

Considering the way of administration – nasal – of the virus, as well as of the therapeutical agent, and the fact that the most serious wounds have been noticed at the level of the respiratory tract, we must refer to them first.

In the control group "A", inoculated with phosphate salt buffer solution, no structural modifications were observed. The lung parenchyma showed collapses of the alveola on limited areas, as a consequence of the functional characteristics existing during the usual respiratory activity.

In the control group "B", to which the parainfluenza virus no. 3 was inoculated, the dominant wound is the inflammatory one (in 100% of the cases), namely the thickening of the alveolar septa, with an incidence of 60%, due mainly to the lympho-histio-macrophagocitary cytoinfiltrate, spread on small areas, especially in the peribronchiolar area. In these areas, usually even the alveolar epithelium has higher cells. In 40% of the cases, a more serious wound than the previous one may be noticed – the lymphohistiocytary zonal interstitial bronchopneumonia, which has no tendency to occupy certain topographic positions in the parenchyma.

In the control groups "C" and "D", to which the therapeutical product was administered, no organized inflammatory processes were noticed in the parenchyma. Instead, we noticed, in both these groups, a thickening of the inter-alveolar septa, mainly as a consequence of the stasis hyperemia, and which is more obvious in group "D", to which the therapeutical agent was administered in two doses, but without an alteration or cytoinfiltrative reaction. In these control groups, we must mention a reaction of the walls of the blood vessels, with an incidence of 16.7% in group "C" and of 33.3% in group "D".

As regards the histochemical image, in the control group "B", inoculated with virus, the AMPS are present (+) in 33.3% of the cases and are mainly situated in the apical area of the bronchiolic epithelium. No LT were noticed. As for the control groups to which the therapeutical product was administered, in group "C" we noticed an obviously positive reaction (++) in 5% of the cases, for the AMPS which are present at the apical pole of the bronchiolic epithelium. As for LT, the reaction was weakly positive (+) in 33.3% of the cases, also at the level of the bronchiolic epithelium. In group "D" to which the therapeutical product was administered in two doses, the reaction for AMPS was positive (+) in 33.3% of the cases at the apical pole of the bronchiolic epithelium. For LT the reaction was weakly positive (+), with an incidence of 33.3% in the bronchiolic epithelium, of 16.7% in the pneumocytes and of 16.7% interstitially in the macrophages.

The histoenzymological reactions have the following incidence: in group "B", the SDH is negative and the LDH is positive (++) in 16.7% of the cases in the bronchiolic epithelium; in the alveolar epithelium, it is (++) in 33.3% of the cases and (+) in 16.7% of the cases. In group "C", the SDH is (+) in 33.3% of the cases in the bronchiolic epithelium; the LDH is (++) in 16.7% of the cases and (+) in 16.7% of the cases in the alveolar epithelium and (+) in 16.7% of the cases in the bronchiolic epithelium. For group "D", the SDH was negative and the LDH was (+) in 16.7% of the cases in the bronchiolic epithelium.

As regards the tracheal wounds present in the control groups, considering the seriousness of the morphopathological image, we mention statistically the following incidence: in group "B" – necrotic tracheitis, in 16.7% of the cases; in group "C" – catar, in 33.3% of the cases; and in group "D", also catar, in 16.7% of the cases.

In the experimental groups, at the level of the respiratory tract, the wounds had numerous variations from the point of view of their frequency, aspect and characteristics.

In group "E", in the lung parenchyma, limited areas with thickened interalveolar septa were noticed as a consequence of the cytoinfiltrate, which also includes rare macrophages (Fig. 1). This type of wound appeared in 66.7% of the cases, of which 16.7% also suffered from a slight hypertrophy of the blood vessels mean, in the vicinity of which a rare lymphohistocytary cytoinfiltrare was present on very reduced tissue surfaces. In this model, the AMPS are obviously positive (++), in 33.3% of the cases, at the level of the apical pole of the bronchiolic epithelium, and the LT is weakly positive (+) in 16.7% of the cases. From the histoenzymological point of view, the SDH is (+) at the level of the alveolar epithelium in 33.3% of the cases, the LDH is (++) in 33.3% and (+) in 16.7% of the cases, in the alveolar epithelium, and (++) in 16.7% of the cases in the bronchiolic epithelium.

As regards the trachea, the most frequent wound is the denudation of the epithelium cilia, which appeared in 33.3% of the cases.

In the case of group "F", in 33.3% of the cases, a slight thickening of the interalveolar septa was noticed on small areas, due to a rare cellular infiltrate, made of lympho-histio-macrophagocytes. In 16.7% of the cases, we noticed a slight thickening of the interparieto-alvelolar space, situated mainly at the perivascular level, as a consequence of the hyperemia and of the oedema. A bronchiolic catar was present in 16.7% of the cases. In 50% of the cases, the AMPS (+) were noticed at the level of the apical pole of the bronchiolic epithelium, under the shape of a strip. The Scherlach colouring for emphasizing the total lipids was negative. The SDH was present (++) at the level of the alveolar epithelium, in 16.7% of the cases, and the LDH (++) in 16.7% of the cases in the bronchiolic epithelium.

At the level of the trachea, the main wound was the denudation of the epithelium cilia in 66.7% of the cases.

In group “G”, in certain areas of the lung, without a strictly localized topography, in 33.3% of the cases, we noticed a thickening of the interalveolar septa, due to the lympho-histiomacrophagocitary cytoinfiltrate and to the oedema which appeared mainly at the peribronchiolar level (Fig. 2). In 16.7% of the cases, on small areas, we noticed a diffuse lympho-histiomacrophagocitary interstitial bronchopneumonia, in which the exudative factor, the oedema, has an important role, as well. The AMPS are present (++) in 33.3% of the cases, under the shape of a strip, at the apical pole of the cells of the bronchiolic epithelium. The LT is also present (+), in 33.3% of the cases, in the macrophages in the alveolar interstitial space. The SDH was not present, while the LDH was (++) in 83.3% of the cases, in the alveolar epithelium, as well as in the bronchiolic one.

In this experimental model, 50% of the cases showed a denudation of the cilia of the tracheal epithelium.

In group “H”, on small areas in the vicinity of the bronchioles, in 33.3% of the cases, a lympho-histio-macrophagocitary bronchopneumonia was noticed, while in 16.7% of the cases, we noticed the thickening of the interalveolar septa, especially in the vicinity of the bronchioles, mainly due to the stasis hyperemia. The AMPS are present, also in the shape of a strip, at the apical pole of the bronchiolic epithelium, in 33.3% of the cases. The LT was noticed in the interstitial space, (+) in the macrophages, in 16.7% of the cases. The SDH was (+) in the alveolar epithelium, in 33.3% of the cases and the LDH was (+) in the alveolar epithelium, in 33.3% of the cases, and (++) in the bronchiolic epithelium in 16.7% of the cases.

As for tracheal wound, the denudation of the epithelium cilia has the highest frequency, namely 83.3%.

In the case of group “I”, in the lung, we found areas in which the parietoalveolar space was thickened due to hyperemia and to a reduced number of macrophages (Fig. 3), in 33.3% of the cases. In this group, no AMPS and LT were noticed in the lung structures. The SDH and LDH were (+) in the alveolar epithelium, in 33.3% of the cases.

The tracheal histo-pathological modifications, with a frequency of 100%, are due to the denudation of the epithelium cilia.

In group “J”, in the lung parenchyma, in the vicinity of the blood vessels, a slight thickening of the inter-parieto-alveolar space was noticed, which in 50% of the cases was due mainly to the hyperemia and the oedema, while in 16.7% of the cases, the hyalinosis of the blood vessels mean was also present. No AMPS and LT were noticed. The SDH was (+) in the bronchiolic epithelium, in 66.7% of the cases, while the LDH was (++) in the alveolar as well as in the bronchiolic epithelium, also in 66.7% of the cases.

The tracheal morpho-pathological image includes either the denudation of the epithelium on the basal membrane, or only the denudation of the epithelial cilia, in 33.3% of the cases.

As for group “K”, in 33.3% of the cases, in the pathostructure of the lung, we noticed a lympho-histio-macrophagocitary interstitial bronchopneumonia, which covers only limited areas, as well as a slight thickening of the interalveolar septa, due to the same cytological elements presented in the previous groups, which are rare, as well as to the hyperemia and to a relative exudation. The AMPS are (+) in 33.3% of the cases, appearing under the shape of a thin strip, at the apical pole of the bronchiolic epithelium, while the LT are absent. The SDH is (+) in 16.7% of the cases, in the alveolar epithelium, and the LDH is (++) in the alveolar epithelium, in 33.3% of the cases, and (+) in the bronchiolic epithelium, also in 33.3% of the cases.

At the tracheal level, the dominant wound is the denudation of the epithelial cilia, in a percentage of 66.7%.

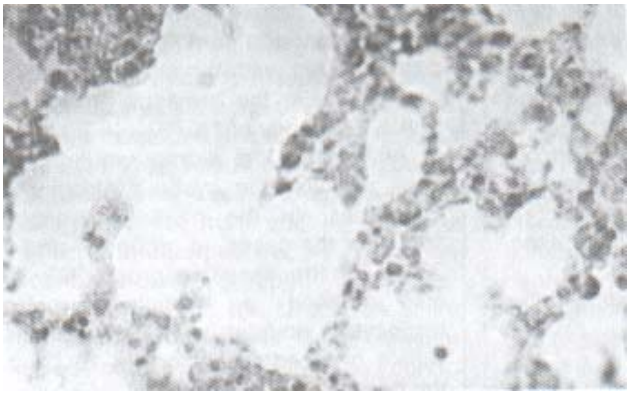

Fig. 1 – The Experimental Model Pl 3 + T1 (3h) – Thikened inter-parieto-alveolar septa due to the cytoinfiltrate which also includes rare macrophages. Col. HE x 400

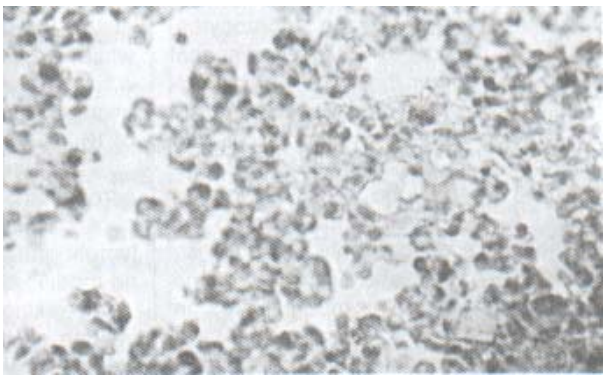

Fig. 2 – The Experimental Model Pl 3 + T1 (24 h) – Thickened inter-parieto-alveolar septa due to the lympho-histio-macrophagocitary cytoinfiltrate and to the very slight oedema. Col. He x 400

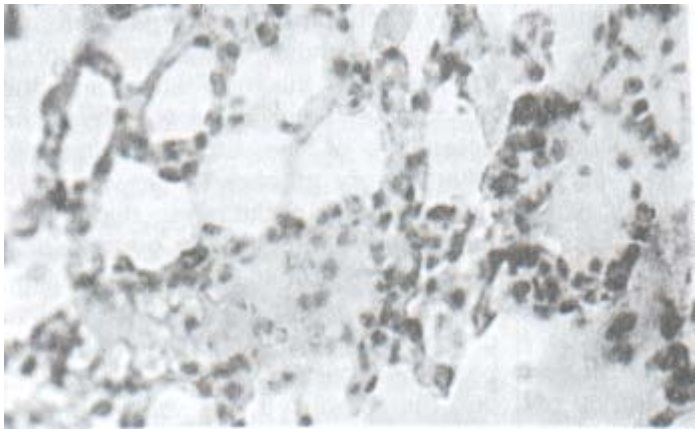

Fig. 3 – The Experimental Model Pl 3 + T1 (24 h) + T2 (48 h) – Thickened inter-parieto-alveolar septa due to hyperemia and to the presence of a reduced number of microphages. Col. HE x 400

The I.F. examination, effected at the level of the lung parenchyma, was negative in groups “A”, “C”, and “D”. In group “B”, the reaction was (++) in the interstitial structures and in the bronchiolic epithelium in 100% of the cases. In the experimental groups, the reactions for I.F. had various aspects: in group “E”, a slight interstitial fluorescence (+), in 16.7% of the cases: in group “F”, a fluorescence (+) present in the alveolar epithelium and in the interstitial space in 33.3% of the cases; in group “G”, the reaction was negative: in group “H”, the fluorescence was very slightly (+) at the surface of the bronchiolic epithelium and spreaded in the interstitial space, in 33.3% of the cases; in group “I”, a slight fluorescence (+) spreaded in the interstitium, in 16.7% of the cases: in group “J”, fluorescence (+) in the interstitium and fluorescent bordering at the surface of the bronchiolic epithelium, in 33.3% of the cases; in group “K’, a slight fluorescence (+) in the interstitium, in 33.3% of the cases.

Morpho-pathological modifications in other organs

In the control groups, in the liver parenchyma, the following wounds were noticed: in group “B”, a stasis congestion, in 16.7% of the cases and a vacuolar dystrophy of the hepatocytes, with a peri-centro-lobular disposal which covers one third of the lobe surface, in 16.7% of the cases; in group “C”, the vacuolar dystrophy of the hepatocytes is present in the peripherical third of the lobe in 33.3% of the cases. At the level of the kidney, the wounds were noticed only in group “B”, respectively the cystic dystrophy of the tubes, especially in the medular area, in 16.7% of the cases. In the spleen, we noticed the presence of the megakariocytes, with a frequency of 2-4 cells per microscopic field, in 16.7% of the cases, both in group “C” and “D”; in group “C”, we also noticed the hypertrophy of the lymphoid follicles in 16.7% of the cases.

In the experimental groups, at the level of the liver, we noticed a vacuolar dystrophy of the hepatocytes, with a diffuse spreading in the lobules, covering up to 50% of their surface, in 33.3% of the cases; in groups “G” and “I”, the same wound covers 30% - 50% of the lobe surface, being situated at the periphery of the lobe, in 50% of the cases; in group “J”, the vacuolar dystrophy of the hepatocytes has a frequency of 50% and a diffuse spreading; in group “K”, we found the same type of hepatocytary wound, with the same spreading as the one in the previous group, but with a frequency of 16.7%.

As regards the kidney wounds, we must mention the cystic dystrophy of the tubes, in the medular area, in 16.7% of the cases, in group “F” and in 33.3% of the cases in group “G”; we also noticed a multiple, lymphohistiocytary nodular interstitial nephritis, with a reduced extension, in 16.7% of the cases.

The morpho-pathological modifications in the spleen were noticed only in group “K” and consist of a hypertrophy of the follicles in 16.7% of the cases, while the presence of the megakariocytes, with an incidence of 1-3 cells per microscopic field reaches the frequency of 16.7% of the cases.

The other examined organs: brain, cerebellum, thyroid, thymus, heart, pancreas, surrenal gland, mediastinal lymphogangliones, ovary, testicle, had no modifications.

Speaking of the results of the observations on the histological, histochemical and histoenzymological modifications on the whole, we must underline the fact that the wounds noticed in the control groups at the level of the respiratory tract and of other organs are different from those in the experimental groups from the qualitative and the quantitative point of view. Thus, the spreading of the broncho-pulmonary inflammatory process is very reduced on the surface of the parenchyma in all the groups in which the therapeutical agent was administered. From the qualitative point of view, in these groups, particular wound elements appear, the cytoinfiltrate is less diversified and more reduced, as well as the alteration, respectively the slight hyalinosis of the blood vessels mean, as in groups “E” and “J”, while the participation of the exudation is more reduced, respectively by means of an oedema.

We must also underline the fact that the pulmonary histo-chemical and histo-enzymological wound image has certain variations, from one experimental group to another, as well as in comparison to the control groups.

At the level of the structures of the pulmonary parenchyma, the IF reaction is obviously positive, in the control group inoculated with the parainfluenza no. 3 virus. In the experimental groups, it is either absent, as in the case of group “G”, or it is very slight and weakly positive in the other experimental groups.

In other organs, in the experimental groups, the vacuolar wound, mainly of the hepatocytes, predominantes in comparison to the proliferative one, and only at the level of the spleen, we may find a hypertrophy of the lymphoid follicles and the presence of the megakariocytes.

Therefore, the elements described draw the attention on the characteristics of the morphopathological modifications resulted after the application of the therapy in the experimental infection with the parainfluenza no. 3 virus.

Discussions

The nasal infestation of the experimental animals with the parainfuenza no. 3 virus and the nasal application of the therapeutical product produces certain wounds, localized mainly at the level of the respiratory tract and which have been observed with the methods applied.

In the control group inoculated with virus, the pulmonary inflammatory wound is well organized, has a high frequency, covers the entire group and has serious forms, such as lymphohistiocytary broncho-pneumonia, in almost half of the animals in the group. In the experimental groups, the wound was found only in the models in which the virus was administered after the therapeutical agent. Under these conditions, it no longer has the same incidence on the respective animals and the participation of the infiltrative elements and of the exudation is slight. In a reduced number of cases (16.7%), the broncho-pneumonia also appears in the group in which the treatment was applied 24 hours after the administration of the virus.

A particular reaction takes place in the inter-alveolar interstitium, in all the groups studies, regardless of the model used. Thus, we may notice the participation of the infiltrative elements together with the oedema, fact which was also described in other reports (CHANOCK, 1990). We must also underline the fact that the cellularity, as well as the exudates, are reduced and sometimes slight, without a tendency to concentration or to nodular organization, extension or spreading. Without being a rule, many times, the thickening of the alveolar walls takes place in the perivascular area. Not rarely, this thickening is due to hyperemia, especially in the groups in which the therapeutical agent was administered before the virus. Seen as a whole, this image suggests the fact that the interstitium, due to its morphology and functionality, tends to limit the reaction of the tissues.

When it reaches the inter-parietoalveolar space, the therapeutical agent is probably conjugated, metabolized and even used and then, by means of a particular clearance process, it is removed from these structures. This is suggested by the fact that the flavonoids in propolis are natural products (BELADI et al., 1977; VECKENSTEDT et al., 1978) used in the daily diet of man animals and that they do not create great problems from the point of view of their metabolization (LANDOLFI et al., 1984).

The reaction of the walls of the blood vessels in the interstitium, though it is sometimes slight or hardly noticeable, has an echo in the control groups inoculated with the therapeutical agent – namely the hypertrophy or sometimes the hyalinosis of the mean of the blood vessels – as well as in the experimental groups – namely the dystrophic wound, present in the group in which the therapeutical agent was administered before and after the inoculation of the virus and in which it was noticed in 50% of the cases. The existence of a certain tropism of the flavonoids towards the vascular structures is possible, this fact being noticed by the presence of the morphological image in the control groups in which no virus was administered; respectively, we must underline the hypertrophy of the mean. In the experimental groups, the vascular reaction was the hyperemia, or the oedema as well, in the appearance of which the activity of the virus may also participate. Also, in the case of the dystrophic aspect, the hyalinosis of the mean, it is possible

that the two factors, the virus and the therapeutical product, may have a synchronous action in the motivation of the installation of the wound.

The histo-chemical image, though different in the control groups in comparison to the experimental ones, does not seem to evolve at significant levels, neither when the virus was singularly inoculated, nor when the therapeutical agent was also singularly administered, or in the experimental groups in which the administration protocols varied.

Considering this fact, in the work model we used, respectively in the acute experiment, we may suggest that the possible distinct or very significant reaction was not possible to be evaluated and that the thesaurization of the AMPS or of the LT may be in connection with the factor “time”, but especially with the factor virus + therapeutical agent, which may induce the histochemical image in progression, towards the image described and in which the therapeutical factor seem to inhibit these accumulations due to its general anti-inflammatory activity.

From the histo-enzymological point of view, we may mention the implication in the pathological processes of the two enzymes studies, considering the fact that *in vivo* the virus has been in contact with the therapeutical factor. If in the control group to which the virus was inoculated singularly the percentages of the animals in which the LDH was particularly obvious were relatively small (16.7%), in the case of the experimental groups, they were significantly higher. Thus, for example, the LDH was of 83.3% in group “G”, in the alveolar, as well as in the bronchiolic epithelium, fact which seem to be due to the increase of the surfaces of the cellular biological membranes which come into contact with the administered product. This image allows us to draw the attention – as other authors did – on the fact that the cell metabolism is intensified through the Embden-Meyerhoff cycle and then through the Krebs cycle of tricarboxylic acids (HODNIC et al., 1988).

By comparing the topographic position occupied by these enzymes in the experimental groups, at the level of the lung structures, we may notice the presence of the SDH, especially at the level of the alveolar epithelium, then, more reduced in the bronchiolic epithelium and absent in the interstitium, while the LDH is present in an almost equal manner in the alveolar and the bronchiolic epitheliums, from the point of view of the frequency and the intensity of the reactions, and insignificantly in the interstitium. This statistical-topographic image of these oxido-reductive enzymes seems characteristic for the models we imagined. Other authors speak about the distribution of these enzymes depending on the specific of the experiment followed and describe completely different localizations (ESKENASY et al., 1976; ESKENASY et al., 1977). Considering the possibility of an imaginary graphic recording of the studied enzymes in relationship with the tissue, by using the ordinate-abscissa system, it seems that the suggested graphs would overlap.

As a consequence of the administration of the therapeutical product, regardless of the moment of the virus inoculation, in general, the lung parenchyma showed – in the experimental groups – inflammatory modifications with a limited surface. The thickening of the inter-alveolar septa, with a diffuse or perivascular distribution, without any particularities of strict localization, is probably due to certain local physiological characteristics or to the dispersion of the therapeutical product in the pulmonary parenchyma.

The interstitial parieto-alveolar cellularity, marked by lymphocytes, histiocytes and macrophages, is induced by the quality and quantity of the antigenes (DUNGWORTH, 1985; HIRSCH, 1984) which produce an obvious reaction of hypersensitivity which will probably facilitate the organization of these elements (TAPU, 1977).

The therapeutical product, through its active part, the flavonoids, is involved in the cytological reactivity whose quality seem to depend on this characteristic (HODNIC et al., 1988). The variations of the enzymatic equilibrium we mentioned above, probably the local enzymatic movements and those of the Embden-Meyerhoff and Krebs cycles, may induce the state of sensitivity to antigens, but also the patho-structural image by means of cytoinfiltrate and exudation. The aspects we presented above seem to offer plausible explanations for the histo-chemical-enzymatic image, but they are similar to other models imagined under rather similar conditions (PETICA et al., 1994). In this respect, probably the therapeutical agent produces certain modifications of the elaboration of the oxygen radicals in the mitochondria with results that seem to limit or even reduce and recover the parieto-alveolar interstitial infiltrate (PETICA et al., 1994).

The electro-chemical characteristics of the flavonoids (HODNIC et al., 1988) may suggest the fact that they may be involved in the recovery of the oedema in the inter-alveolar space, activity which probably represents the beginning of the functional recovery of the tissues (PETICA et al., 1994). In this respect, there probably exists an indirect effect on the vascular structures in the lung, as a consequence of the release of the local chemical mediators, respectively, through the activity of degranulation of the mastocytes (LANDOLFI et al., 1984; PETICA, 1994). Thus the hyperemia appears, as well as the hypertrophy of the blood vessels mean in the control groups without virus, while in some of the experimental groups, the hyalinosis of this mean takes place, probably as a consequence of the metabolic disturbances at the level of this structure (PETICA et al., 1994).

As for the histo-pathological picture in the other organs, we must mention the constant reaction to the action of the virus, which is manifesting itself by means of the denudation of the cilia in the tracheal epithelium, fact pointed out by other observations as well (LANDOLFI et al., 1984; TYLER, 1990). Probably, a synchronous reaction, virus + therapeutical agent may be attributed to the vacuolar dystrophy of the hepatocytes, especially in the experimental models in which the therapeutical agent was administered 24

hours after the inoculation of the virus, but also in the reserved situation. Also, the same thing was noticed in the groups in which two sessions of treatment were applied. It seems that this relatively transitory and rather unimportant relation is more a reaction to the viral aggression, the cumulated action of the virus and the therapeutical agent being also possible and in this case the aspect of the morphological, hepatic image may be the result.

The structural images in the kidneys concern more the vascular morphopathology and we attribute them the same combined actions, virus + therapeutical agent. They have a relatively small incidence and their aspect also suggests possibilities of recovery. The kidney dystrophic wounds also seem to be the result of the action of the same couple of factors.

The reactions at the level of the spleen parenchyma are probably due either to the stimulation of certain structures or to the inhibition of the functional processes, aspects represented by hypertrophies or hypotrophies of the lymphoid follicles, and the presence in certain experimental models of the megakariocytes is probably due more to the activity of the therapeutical agent on this strain of cells.

Conclusions

1. The parainfluenza no. 3 virus, strain 739, 2D was intranasally inoculated in mice and, for therapeutical purposes, an aqueous flavonoids extract obtained from propolis by means of refluxation, by an original method was administered through the same way to mice.
2. The therapeutical agent is well tolerated by the organism and seems to have a capacity of penetration through the parieto-alveolar structures.
3. The therapeutical agent has the tendency to limit the pulmonary inflammatory process.
4. In the majority of the treated groups, the broncho-pneumonia is absent or has a low incidence, from the point of view of the percentages.
5. At the level of inter-parieto-alveolar thickened interstitium, the therapeutical agent reduces the lympho-histio-macrophagocitary infiltration and the exudation, the oedema, fact which suggests an image of recovery in evolution.
6. The prioritary inoculation of the virus followed by the administration of the therapeutical agent offers an advanced recovery image, especially when the interval between the applications is shorter and the administered doses are more.

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