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Dipeptidyl peptidase IV (DPPIV) activity in the tear fluid as an indicator of the severity of corneal injury: a histochemical and biochemical study

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Summary. Comparative histochemical and biochemical studies on the catalytically active protease Dipeptidyl peptidase IV (DPPIV), have been performed in the rabbit cornea and the tear fluid using a sensitive fluorogenic substrate, Gly-Pro-7-amino-4-Trifluoromethyl Coumarine (AFC). In both normal and experimentally injured corneas, DPPIV activity was detected histochemically and in the tear fluid biochemically. In contrast to the normal cornea where DPPIV activity was absent and in the tear fluid where it was low, during continuous wearing of contact lenses or repeated irradiation of the cornea with UVB rays, slight DPPIV activity appeared first in the superficial layers of the corneal epithelium, while later increased activity was present in the whole epithelium. This paralleled elevated DPPIV activity in the tear fluid. Moreover, during continuous contact lens wear, the increased DPPIV activity in the tear fluid was, in many cases, coincidental with the presence of capillaries in the limbal part of the corneal stroma. After severe alkali burns when corneal ulcers appeared, collagen fragments were active for DPPIV, which was associated with high DPPIV activity in the tear fluid. In conclusion, Gly-Pro-AFC was found to be useful for comparative histochemical and biochemical studies on DPPIV activity in the experimentally injured rabbit eye. Using the method of the tear film collection by a short touch of substrate punches to the respective site of the cornea or conjunctiva we can show that in experimental injuries (wearing of contact lenses, irradiation of the cornea with UVB rays), the damaged corneal cells were the main source for DPPIV activity in the tear fluid. It is suggested that the activity of DPPIV measured in the tear fluid might serve as an indicator of early corneal disorders, e.g. corneal vascularization related to contact lens wear.

Key words: Dipeptidyl peptidase IV, tear fluid, corneal injury

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Introduction

Dipeptidyl peptidase IV (DPPIV) (CD26, EC 3.4.14.5), one of the membrane exopeptidases, is a serine protease that cleaves dipeptides with proline in the penultimate position from the aminoterminus of a polypetide chain. According to Boonacker and Van Noorden (2003), CD26/DPPIV can be considered as a moonlighting protein because it is a multifunctional protein that exerts its different functions depending on cell type and the intra- or extracellular conditions in which it is expressed. For the localization of DPPIV as well as other proteolytic enzymes, particular attention is given to substrates containing the leaving groups 4-methoxy-2-naphthylamide (MNA) and 7-amino-4-trifluoromethylcoumarin (AFC). The MNA substrates are used for histochemical purposes; they yield a colored final reaction product when azo-coupled with a diazonium salt. AFC substrates, which are more sensitive, yield the fluorescent product AFC after enzymatic cleavage of the substrate. In contrast to MNA substrates, AFC substrates are not recommended for histochemical purposes - for (intra)cellular localization - because these substrates are not sufficiently water-insoluble (Smith et al., 1992). They are recommended for incubation in microwells (Immu-Probe techniques) and for the demonstration of banding patterns after gel electrophoresis (enzyme-directed overlay membrane technique). However, Lojda (1996) developed a new histochemical method with AFC substrates for proteases using cryostat sections on semipermeable membranes

and a gel incubation media. This approach strongly limited diffusion, and an acceptable cellular localization was achieved. Lojda (1996) recommended AFC substrates for comparative histochemical and biochemical studies on proteases using the same substrate and for cases in which no other reliable procedure for the localization of the respective enzyme activity was available (e.g. urokinase, plasmin). The biochemical approach is based on the use of filter paper punches containing the substrate on which several ml of tears or other fluids are dropped. The incubation takes place at 37 ºC, and the time of the appearance of the yellowish fluorescence in calibrated punches containing a known concentration of the respective enzyme (Čejková et al., 1992, 1993; Lojda and Čejková, 1993; Čejková and Lojda, 1995; Lojda, 1996; Čejková et al., 1999) is recorded. Smith et al. (1992) used a colorimetric method instead of fluorescence: after the addition of a solution of p-dimethylamino-cinnamaldehyde, a reddish dye is formed by the liberated AFC.

In this paper, we used the described semiquantitative biochemnical method for the investigation of DPPIV activity in the tear fluid using the sensitive fluorogenic substrate Gly-Pro-AFC. The same substrate was also employed for the in situ (in the cornea) detection of DPPIV activity using the method as developed by Lojda (1996). Because until now the substrate Gly-Pro-AFC has not been used previously for the detection of DPPIV activity in the cornea, the histochemical results obtained with this substrate were compared with histochemical results achieved with Gly-Pro-MNA and Fast Blue B in chloroform-acetone- pretreated cryostat sections adherent to glass slides (see Lojda, 1981; Čejková and Lojda, 1986; Smith et al., 1992 for details).

Material and methods

Experimental injuries

Female New Zealand White rabbits weighing 2.5-3.0 kg were used in our experiments. Histochemical as well as biochemical investigations of DPPIV activity were performed in normal eyes as well as in eyes under various experimental conditions (contact lens wear, repeated irradiation of the cornea with UVB rays, alkali burns). The procedures used in this communication were consistent with the ARVO resolution on the Use of Animals in Research, according to the World Medical Association Declaration of Helsinki, Finland, 1964 and revised by the World Medical Assembly in Hong Kong in 1989.

Contact lens wear

The soft contact lenses used in this study were not contact lenses for humans, however contact lenses specifically manufactured for rabbit eyes as follows : 1-vinyl-2-pyrrolidone with 2-acetoxyethyl methacrylate, oxygen permeability, DK, 35, average central thickness 0.15 mm, diameter 13.5, water content 68.5%. Calculation of soft contact lens parameters after measuring the eye geometry, as well as the manufacture of contact lenses for rabbits by turning, was done in the Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Prague, CR. The eye lids of the rabbits were slightly distended and sterile contact lenses were applied on the corneas of both eyes. The lenses remained continuously on the eyes of the first group (8 rabbits) for 2 days, in the second group (8 rabbits) for 4 days and in the third group (8 rabbits) for 6 days. After these time intervals, the contact lenses were removed. In the third group of rabbits the level of DPPIV activity in the tear fluid was measured (on day 6) in both eyes using a semiquantitative biochemical method. For histochemical and histological examination, rabbits of all three groups were employed. After the end of each time interval of continuous contact lens wear, the rabbits were sacrificed by i.v. thiopental anesthesia (thiopentalum natricum, Spofa, Praha). However, prior to thiopental anesthesia, the rabbits were premedicated with an i.m. injection of Narkamon (ketaminum hydrochoridum, Spofa, Prague, 5%, 1ml/1kg weight), and Rometar (Xylazinum hydrochloridum, Spofa, 0.2%, 0.2ml/1kg weight).

Repeated irradiation of the rabbit eye with UVB rays

The rabbits were anesthetized by an i.m. injection of Narkamon and Rometar (Narkamon 5%, 1ml/1kg weight + Rometar 0.2% 0.2ml/1kg weight). Both eyes of 8 rabbits were irradiated. The eyes were open and the corneas (the other parts of the eye were covered with a sterile cotton gauze) were irradiated from a distance of 0.15 m with a Bioblock UV lamp (Scientific, Illkirch, Cedex, France), which generates UV rays of 312 nm wavelength (UVB rays), for 5 min once a day, for 6 days. On day 6 DPPIV activity was measured in the tear fluid and the rabbits were sacrificed by a thiopental anesthesia (prior to thiopental anesthesia rabbits were premedicated with an i.m. injection of Rometar and Narkamon). Immediately after the death of the animals, the corneas were examined histochemically and histologically.

The burning of corneas with strong alkali

The rabbits were anesthetized by an i.m. injection of Narkamon and Rometar (Narkamon 5%, 1ml/1kg weight + Rometar 0.2% 0.2ml/1kg weight). Both eyes of 8 rabbits were burned. The eyes were proptosed and 1.0M NaOH was applied on the cornea using a 9 mm-diameter plastic tube, for 15 s. After the quick removal of alkali by a cotton swab the eyes were rinsed with tap water. The animals were left without any treatment for 28 days. On day 28 the DPPIV activity was measured in the tear fluid, and the animals were sacrificed by i.v. thiopental anesthesia (before thiopental anesthesia rabbits were

premedicated with an i.m. injection of Rometar and Narkamon). The corneas were examined histochemically and histologically.

Histochemical examination

The eyes of normal rabbits served as controls. The normal and experimentally injured rabbit corneas were employed. The anterior eye segment were quenched in light petroleum chilled with an acetone-dry ice mixture, and corneal sections were cut in a cryostat (Jung, Leica Instruments GmbH, CM 1900, Heidelberg, CM 1900, Heidelberg, Germany).

DPPIV activity was detected by two methods. With the first method - an azo-coupling reaction - Gly-Pro-4-methoxy-2-naphthylamide (MNA) and Fast Blue B (FBB) (Bachem, Feinchemikalien, Bubendorf, Switzerland) were used. The method was performed on cryostat sections on slides of unfixed corneas post-fixed with a cold chloroform-acetone mixture (see Lojda, 1981; Čejková and Lojda, 1986 for details). With this method, sections incubated in the incubation media without substrate served as controls. With the second method, Gly-Pro-7-amino-4-Trifluoromethyl Coumarine (AFC) (Enzyme Systems Products, Dublin, CA, USA) was employed in the procedure as described by Lojda (1996) for proteases. The method was performed on unfixed cryostat sections on semipermeable membranes with a gel incubation media. Briefly: 0.9 ml of 0.1 M Tris-HCl buffer, pH 7.4-7.8, was added to 0.1 ml of the dissolved substrate. The solution was mixed with 1ml of 2% solution of Bacto-Agar (Difco, Detroit, Mich., USA) prepared at 60 ºC in the respective buffer. After careful mixing the media was poured into the semipermeable membrane vessels and left to gelify in a refrigerator. The incubation at 37 ºC lasted for several hours. Sections incubated in the gel incubation media without substrate served as controls.

Histological examination

In all experimental groups some cryostat sections were post-fixed in formol-calcium chloride and stained with Haematoxylin-eosin.

Biochemical investigation

The assessment of DPPIV activity (calibrated punches). Filter paper punches (5 mm diameter) were put into the substrate solution, then removed, transferred onto a glass plate and allowed to dry. The substrate solution was prepared as follows: 1 mg of substrate (Gly-Pro-AFC) (Enzyme Systems Products, Dublin, CA, USA) was dissolved in four drops of DMF (N,N,dimethlyformamide, Sigma) and 1 ml of Tris-HCl buffer, pH 7.8 was added. For the semiquantitative determination of DPPIV activity, solutions containing DPPIV (from porcine kidney, vial containing 1.5 units, Sigma) at various concentrations (0.75, 0.375, 0.187, 0.094, 0.047, 0.023 units/ml Tris-HCl, pH 7.4-7.8) were prepared. (Sigma, unit definition: one unit will produce 1.0 mole of p-nitroaniline from Gly-L-Pro p-nitroanilide per min in 100 mM Tris-HCl at pH 8.0 at 37º C). Five μl of each sample were dropped onto dry substrate punches ("calibrated punches"). Punches were then incubated in a wet chamber with a thermostat (37º C) and observed under UV light using a hand fluorescent lamp (UVGL-25, manufactured by CVP, Inc., San Gabriel, CA, USA) as follows: at 1 min intervals during the first 5 min and at 5 min intervals thereafter. The time of the first appearance of yellowish fluorescence was recorded. This procedure was performed with 5 DPPIV specimens (from porcine kidney, Sigma, concentrations 0.75 – 0.023 units/ml Tris-HCl buffer, pH 7.4-7.8). With each specimen, two measurements at all the above mentioned concentrations were performed.

The DPPIV activity in the tear fluid was measured in the following way: substrate punches were soaked with the tear fluid either by dropping 5 ml of the tear fluid collected with a micropipette (for statistical evaluation) or by a short touch of the substrate punch to the respective site of the cornea or conjunctiva. This mode of the tear film collection enable us to examine the cell source for DPPIV activity. Then substrate punches with the tear fluid were incubated in a wet chamber with a thermostat (37 ºC) together with calibrated punches. The appearance of positive fluorescence in punches with the tear fluid was compared with the appearance of positive fluorescence in calibrated punches.

Statistics

From the data (the measurements of DPPIV activity in the tear fluid of normal eyes and eyes in which corneas were experimentally injured) the following model of calibration curve was selected : y = exp(exp(a+b.log((log(3)/log(x)/log(2)))), where y is the time of the appearance of yellowish fluorescence (enzyme positivity) in seconds and x the enzyme concentration in units/ml.

The mathematical formula of the curve was estimated from the measured data in such a way that the data were transformed to reach the homoscedascity and the parameters of the curve were calculated using the method of the least squares. The estimated parameters of the calibration curve are a=1.4594 and b=0.4078. The calibration curve (Fig. 2) can be used for the prediction of a DPPIV concentration based on the measured time of the appearance of the positive fluorescence. The marked points on the calibration curve belong to the measured times of the appearance of the positive fluorescence for substrate punches (with 5 ml of the tear fluid): ten punches of normal rabbit eyes, ten punches of eyes with contact lenses (measured on day 6 of the continuous wearing of contact lenses), ten punches of UVB irradiation (measured on day 6 of repeated irradiation of the cornea) and ten punches of corneal ulcers (measured on day 28 after severe alkali burns) were examined (Fig.

2). Thus, we could use the calibration curve for estimating the concentration from the measured time. Moreover, we can calculate the lower and upper bounds of the 95% confidence interval for the concentration.

Results

Histochemical and histological examination

Both substrates (Gly-Pro-MNA and Gly-Pro-AFC)

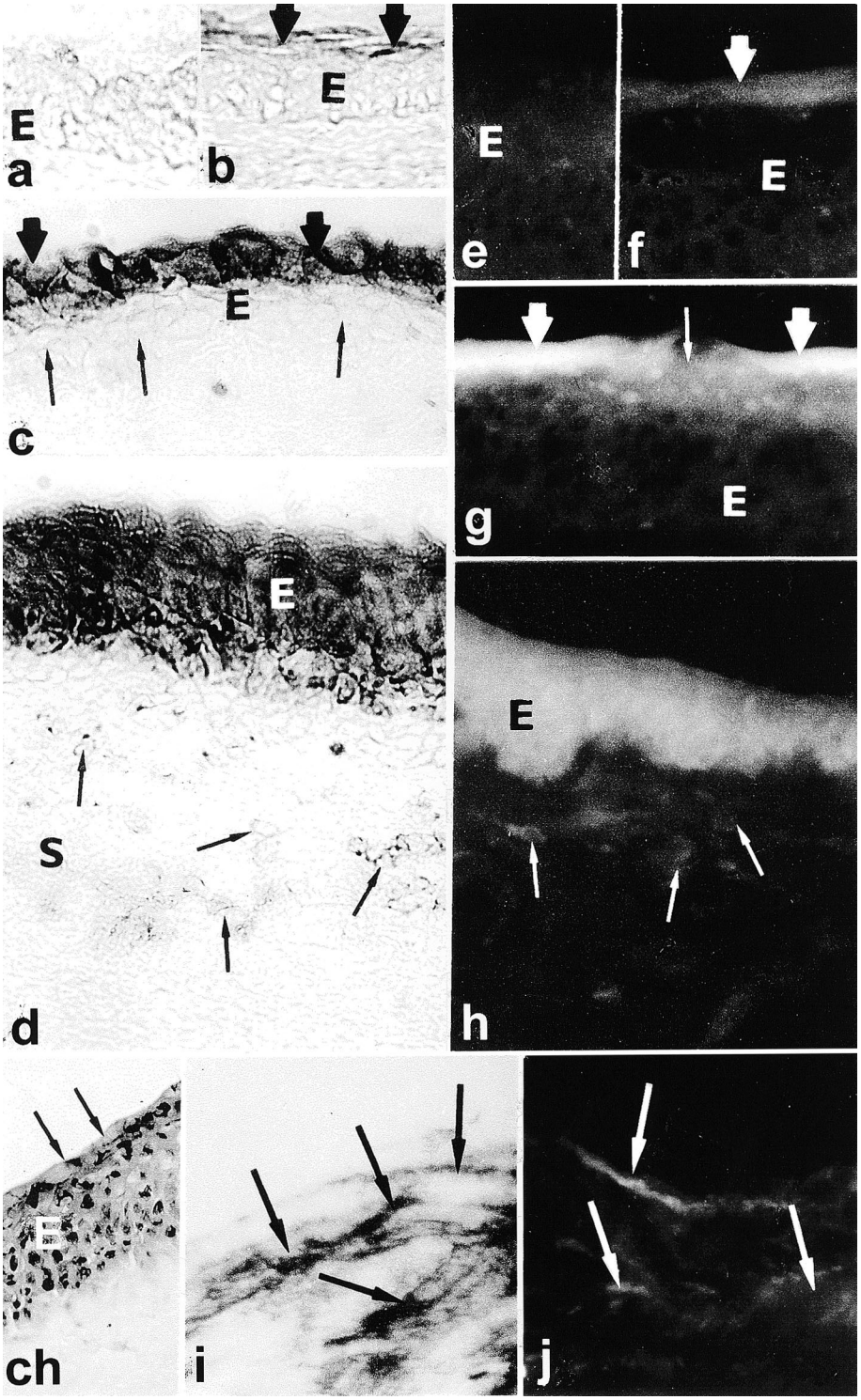

Fig. 1. Histochemical results on Dipeptidyl peptidase IV (DPPIV) in the normal and experimentally injured rabbit cornea using Gly-Pro-MNA **(a, b, c, d, i)** and Gly-Pro-AFC **(e, f, g, h, j)** as substrates. Histological pattern of the corneal epithelium after 6 days of the continuous wearing of contact lenses (ch). **a, e.** Normal corneal epithelium (E). DPPIV activity is absent in the corneal epithelium. **b, f.** Two days of continuous contact lens wear. A slight DPPIV activity appears in the superficial layers of the corneal epithelium (arrows). **c, g.** Four days of continuous contact lens wear. Increased DPPIV activity is also present in the deeper parts of the corneal epithelium (arrows). **d, h.** Six days of continuous contact lens wear. The whole corneal epithelium (E) is active for DPPIV. In the corneal stroma (S), corneal vascularization is visible (arrows point to capillaries bearing DPPIV activity). **ch.** On day 6 of the continuous wearing of contact lenses the superficial layers of the corneal epithelium (E) are slightly damaged (arrows). **i, j.** Day 28 after severe alkali burn. Collagen fragments in the damaged corneal stroma are highly active for DPPIV. x 140

revealed similar histochemical results in the cornea. Using AFC substrate, an acceptable cellular localization was achieved. In Fig1. a-i, DPPIV activity was detected using Gly-Pro-MNA. In Fig. 1. e- j, DPPIV activity was localized with Gly-Pro-AFC. In the normal corneal epithelium, DPPIV activity was absent (Fig. 1a, e). After 2 days of continuous wearing of contact lenses, slight DPPIV activity appeared in the superficial layers of the corneal epithelium (arrows) (Fig. 1b, f). After 4 days of continuous wearing of contact lenses, increased DPPIV activity was located not only in the superficial epithelial layers but also in the deeper portion of the epithelium (arrows) (Fig. 1c, g). The continuous wearing of contact lenses for 6 days evoked the presence of DPPIV activity throughout the whole epithelium (Fig.1, d, h). In Fig. 1d,h arrows point to capillaries present in the limbal region of the corneal stroma. Also, after repeated irradiation of the cornea with UVB rays for 6 days, DPPIV activity was present throughout the whole corneal epithelium. During corneal ulceration after severe alkali burns (day 28 after alkali injury), collagen fragments present in the residual part of the damaged corneal stroma showed DPPIV activity (arrows), (Fig. 1i,j).

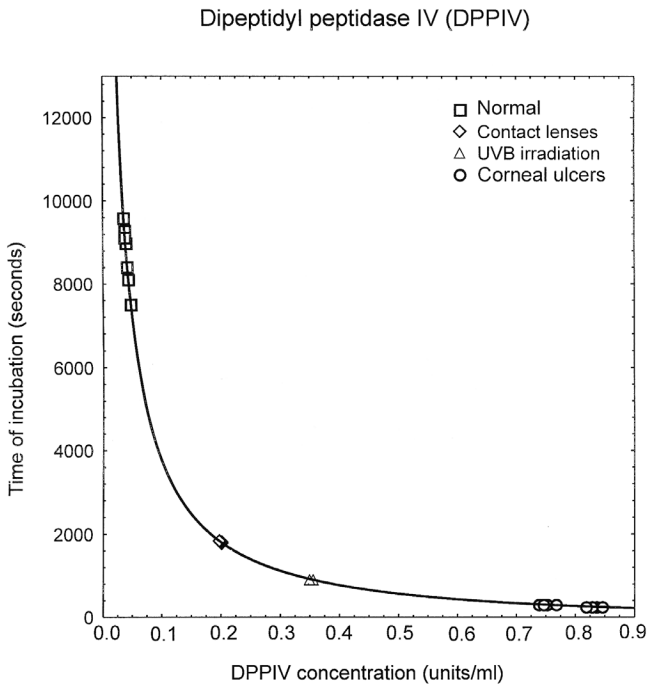

Fig. 2. Calibration curve of Dipeptidyl peptidase (DPPIV) activity in the tear fluid of normal rabbit eyes (Normal), eyes after the continuous wearing of contact lenses for 6 days (Contact lenses, DPPIV activity measured on day 6), repeated irradiation of the rabbit eye with UVB rays for 6 days (UVB irradiation, DPPIV activity measured on day 6) and corneal ulceration after a severe alkali burn (Ulcers, DPPIV activity measured on day 28 after severe alkali injury). The calibration curve was based on the appearance of enzyme positivity (yellowish fluorescence) at various time intervals of substrate punches with the tear fluid (5 μl) collected by micropipette (time in seconds). The enzyme positivity was examined by comparing the appearance of yellowish fluorescence in substrate punches under investigation with the appearance of the positive fluorescence in "calibrated punches" (substrate punches with 5 μl of DPPIV, Sigma, in various concentrations, units/ml). Thus DPPIV concentrations were fixed and therefore belonged to the independent variable x in the regression model. The time of the appearance of the positive fluorescence was examined for each DPPIV concentration and therefore the time was dependent variable y in the regression model). In the normal eyes DPPIV activity was low in the tear fluid. DPPIV activity was increased in the tear fluid (in ascending order) after the continuous wearing of contact lenses, irradiation of the cornea with UVB rays and corneal ulcers after severe alkali burns.

The appearance of DPPIV activity in the corneal epithelium during continuous wearing of contact lenses (and also changes in activities of some other enzymes, see Čejková et al., 1988) appear earlier than morphological disturbances. The morphological pattern of the corneal epithelium remain normal up to day 5 of the continuous wearing of contact lenses and on day 6-7 superficial layers of the corneal epithelium are destroyed (Fig. 1c,h). However, the places where superficial epithelial layers are damaged are better seen using the silver impregnation technique in the stripped corneal epithelium (see Čejková et al., 1988, 1992 for details). After the repeated irradiation of the cornea with UVB rays for 6 days superficial layers of the corneal epithelium are lost. After severe alkali burns the cornea is dramatically damaged (nearly the whole cornea is necrotic) and on day 28 after the alkali injury deep stromal ulcers appear; some corneas are perforated (see Čejková and Lojda, 1988; Čejková et al., 1988 for details).

The appearance of corneal vascularization related to continuous contact lens wear was as follows : after 2 days of contact lens wear, corneal vascularization was not observed in any case; four days of continuous contact lens wear evoked the appearance of capillaries in the corneal stroma in 30% of the corneas; and after 6 days of continuous contact lens wear vascularization of the cornea was observed in 80% of the corneas. The percentage of corneal ulcers after severe alkali burns : on day 28 after, the majority of the corneas were ulcerated (80% of the corneas).

In control reactions (the incubation solutions without substrates) DPPIV positivity was not obtained in any sample.

Biochemical investigation

Results of the measurement of DPPIV activity in the tear fluid are summarized in Fig. 2. DPPIV activity was low in the tear fluid of the normal rabbit eyes. The concentration of DPPIV activity increased in the tear fluid along with increasing corneal damage in ascending order after prolonged wearing of contact lenses for 6 days (contact lenses, DPPIV measured on day 6), repeated irradiation of the eye with UVB rays for 6 days (irradiation with UVB rays, DPPIV measured on day 6) and corneal ulceration after severe alkali burns (corneal

ulcers, DPPIV measured on day 28 after the severe burning of the cornea with alkali).

Discussion

The in situ localization of Dipeptidyl peptidase DPPIV (DPPIV) can be performed at both the protein and activity level. Immunohistochemical studies detecting the protein DPPIV are very important; however, they have the disadvantage that both active and inactive enzyme molecules are localized. Because we want to employ the measurement of DPPIV activity in the tear fluid as an indicator of corneal disorders in humans in the future, we first had to compare (in the rabbit eye) DPPIV activity in the cornea with DPPIV activity in the tear fluid under various experimental conditions. This was the subject of the present study. We compared the appearance and activity of DPPIV in the rabbit cornea (after the repeated irradiation of the cornea with UVB rays, prolonged wearing of contact lenses and severe alkali burns) with the level of DPPIV in the tear fluid. For this reason, a histochemical approach was employed with a sensitive fluorogenic substrate, Gly-Pro-AFC, for the localization of DPPIV in situ (in the cornea) (method recommended by Lojda, 1996). The same substrate (Gly-Pro-AFC) was used for the measurement of DPPIV activity in tear fluid, using the semiquantitative biochemical method described above. This method was previously found to be useful for the measurement of plasmin, urokinase and acid ß-galactosidase in tear fluid (Čejková et al., 1993, 1999; Čejková and Lojda, 1995). It must be pointed out that although various methods exist for the investigation of proteases (and also other enzymes) in body fluids and secretions (including the tear fluid and aqueous humor), the advantage of the method used in this study is its sensitivity and simplicity. It does not require any expensive laboratory equipment (only a thermostat and a hand fluorescent lamp), and therefore it can be used in every ophthalmology department.

Because until now DPPIV activity has not been detected in the cornea (or other eye tissues) using the substrate Gly-Pro-AFC, the results obtained with this substrate were compared with the results achieved with the substrate Gly-Pro-MNA, previously found to be useful for the localization of DPPIV in situ (Lojda, 1981, Čejková and Lojda, 1986, Smith et al., 1992). Similar histochemical results for DPPIV activity were obtained with both substrates in the cornea. In contrast to the normal rabbit cornea, where DPPIV activity was absent and low in the tear fluid, in experimentally injured rabbit corneas a slight DPPIV activity appeared in the superficial layers of the corneal epithelium, and later (together with greater corneal damage) the activity was present in the whole epithelium. This was accompanied by elevated DPPIV activity in the tear fluid. During continuous wearing of contact lenses, in many cases the elevation of DPPIV in the tear fluid was coincidental with corneal vascularization (the appearance of capillaries in the limbal part of the corneal stroma). It was previously found that DPPIV activity is present on the membranes of the capillary endothelium (e.g. Heymann and Mentlein, 1984; Gossrau, 1985; Lojda et al., 1985). Corneal vascularization related to contact lens wear often appears in humans and is very dangerous to the cornea. It appears as a result of a combination of hypoxia and epithelial trauma (Madigan et al., 1990). Leukocyte migration into the corneal stroma and the release of angiogenic factors from these cells may subsequently promote new vessel growth (Efron, 1987). However, it was also found that a homogenate of corneal epithelium is able to provoke corneal vascularization in the absence of leukocytes. Thus, even if the process of vascularization of the cornea is a complex one, the corneal epithelium might play an important role in the process by providing initial signals for vessel activation and growth (Eliason, 1985). It was further found that after severe alkali burns, when corneal ulcers appeared, collagen fragments in the damaged corneal stroma were highly active for DPPIV. This was described by Pahlitzsch and Sinha (1985) in the alkali-burned rabbit cornea. However, as found in these experiments, corneal ulceration after severe alkali injury of the cornea and the appearance of DPPIV activity on collagen fragments were coincidental with highly elevated DPPIV activity in the tear fluid.

In contrast to the eye, where until this study DPPIV activity had not been investigated in the tear fluid, DPPIV activity had been measured in the serum of patients with primary biliary cirrhosis and chronic hepatitis C virus infection (Lakatos et al., 2000). Because DPPIV activity was significantly elevated in serum, the authors suggested that DPPIV might be an indicator for chronic liver injury. Also, Perner et al. (1999) described the clinical utility of serum and urinary DPPIV activity measurements in adult and pediatric patients with hepatobiliary diseases and in liver transplant recipients. Additionally, DPPIV activity was found to be useful as a urinary test for cholestasis in infants. Scherberich et al. (1992) studied the biochemical and immunological properties of urinary angiotensinase A and DPPIV. These authors presumed that these enzymes might be used in clinical practice as markers of renal cell injury. Rao et al. (1990) and Prager et al. (1994) detected DPPIV activity in burn exudates of dermal wounds and also in effusions from patients with chronic otitis media with effusion. The authors suggested that the main source of DPPIV activity in effusions were the cells of the middle ear. In the eye, some enzymes are secreted into tears by the lacrimal gland or can be liberated into tear fluid after damage of the corneal or conjunctival epithelium. However, as described by Van Haeringen and Glasius (1976), enzymes that are secreted by the lacrimal gland might also be present in increased amounts in tears after damage of the corneal or conjunctival epithelium. Because in our experiments only the corneas were injured and the increased DPPIV activity in corneal cells

was related to an increased level of DPPIV activity in the tear fluid, it is suggested that the main source for DPPIV in the tear fluid is damaged corneal cells, mainly epithelial cells. As found previously, increased activities of some other enzymes - enzymes of the plasminogen-activator/plasmin system and of acid ß-galactosidase - in the tear fluid also paralleled the corneal damage (Salonen et al., 1987; Čejková et al., 1989, 1992, 1993, 1999; Barlati et al., 1990; Berta et al., 1990; Lojda and âejková, 1993, Čejková and Lojda, 1995). These studies were performed mainly for therapeutic purposes. The knowledge of increased protease activities in tear fluid has been important for the therapeutic use of enzyme inhibitors which have enabled the healing of corneal wounds, e.g. aprotinin (an inhibitor of plasmin and some other proteases) (e.g. Čejková et al., 1993; Čejková and Lojda, 1995; Karon and Klyce, 2003).

It can be concluded that Gly-Pro-AFC is suitable for comparative histochemical and biochemical studies of DPPIV activity in the rabbit eye. The increase in DPPIV activity in the injured rabbit cornea paralleled the increase of DPPIV activity in the tear fluid. Using the method of the tear fluid collection by a short touch of substrate punches to the respective site of the cornea or conjunctiva it can be shown that during continuous wearing of contact lenses (up to day 6) and also after early irradiation of the isolated cornea with UVB rays, the damaged corneal cells were the main source for DPPIV activity in the tear fluid. During corneal ulceration after alkali burns high DPPIV activity was found in the site of ulcers, however, in such severe injury of the eye also alternate sources for DPPIV must be taken into consideration.

In conclusion, we suggest that the measurement of DPPIV activity in the tear fluid might be useful mainly during wearing of contact lenses because early disorders of the corneal epithelium might be accompanied with corneal vascularization which is dangerous to the cornea. However, for the employment of DPPIV in clinical practice, studies on DPPIV activity in the human eye are necessary. The description of detailed data obtained in humans will be the subject of our next communication.

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