Assessment of potassium and sodium ion concentrations in the vitreous humour of swine isolated eyeballs after organism death

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Abstract

Potassium and sodium ion concentrations were estimated by the flame photometry and potentiometry in the vitreous fluid of isolated porcine eyeballs at time of death and of eyeballs, stored at temperature of 6-8^{0}C during post-mortem intervals: 4, 28, 52, 75, 100, 124 and 148 hours. The increase of K^+ concentration and decrease of Na^+ concentration were proportional to the increasing post-mortem time intervals. The results of the potentiometric measurements of K^+ and sodium ion concentrations were significantly lover, as compared to those after flame photometry. In all the vitreous fluid smears after 124 and 148 hours, Gram (-) bacteria were found. Our results suggest that bacterial infection participates in the variability of K^+ levels. The influence of bacterial infection on the margin of error for the K^+ post-mortem test remains unanswered and needs further studies.

Key words: post mortem interval, vitreous fluid, swine, potassium and sodium ions, bacteria.

Introduction

Precise determination of the post-mortem interval (PMI) remains unsolved up to now. From the year 1959, in legal medicine, popular has been the estimation of PMI effects on the results of vitreous fluid K^+ concentrations. Different, mainly linear, equations that describe the relationship between the vitreous fluid K^+ concentrations and PMI have been developed. Normal vitreous K^+ concentration (3.14-8 mmol/L) and the PMI hour increase coefficient (0.14-0.55 mmol/L) were different in manner, depending on the paper's authors. The human vitreous K^+ level is dependent on different factors, e.g., temperature, agony, and ion determination methods [1, 2, 3]. In the veterinary research, a promising diagnostic method of assessing the cause of animal death is the estimation of the chemical composition of the vitreous fluid [4]. These studies also include an estimation of K^+ and Na^+ ion concentrations. The aim of the study, reported here, was an assessment of K^+ and Na^+ ions by two different analytical methods (flame photometry and potentiometry) in the liquid portion of the vitreous fluid (LPVF) of isolated porcine eyeballs at time of death and stored at temperature of 6-8^{0}C during selected post-mortem intervals.

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Materials and Methods

The eyeballs were collected from pigs, each 100-120 kg of body weight, delivered to butchery during up to 60 min. before death. All the animals were inspected by veterinary control. The animals were struck by electric shock and exsanguinated. The eyeballs, collected immediately after animal death (up to 10 min.), were included into the control group. Each eyeball was covered by a parafilm and aluminium foil, packed in a small plastic container (the cornea on top) and stored at temperature of 6-8^{0}C. Those eyeballs were divided into groups, according to the following post-mortem intervals: 4, 28, 52, 76, 100, 124, 148 hours. The vitreous fluid was collected, acc.to Coe's method [5]. For separation of LPVF, nylon (0.42 μm) filters were employed. Separation control of the gelatinous portion from the LPVF (for ion concentration study) was performed on stained fluid smears, prepared before and after the separation (H&E -for cells, nuclei and other cellular structures or Gram method- for bacteria, microscopy- 100x immersion). The volume of 1.5 -2.0 LPVF was divided into two portions of equal volumes. In each liquid portion volume, Na^+ and K^+ ion concentrations were

Table 1. Average concentration of K^+ and Na^+ (mmol/L) ions in the control group and PIM (in hours) in the liquid portion of the swine vitreous fluid, estimated by flame photometry and potentiometry methods.

	C	4	28	52	76	100	124	148
Potassium Flame Photometry	x = 6.7 SD = 0.85 n = 20 v =12.68%	x = 8.45 SD = 1.12 n = 108 v = 13.25%	X =15.85 SD =1.05 n =36 v = 6.62%	x =22.48 SD =1.48 n = 36 v = 6.58%	x =23.35 SD =1.48 n =36 v = 6.33%	x =26.75 SD =1.96 n = 36 v = 7.32%	x =23.12 SD =4.46 n = 36 v = 19.29%	x = 24.5 SD =3.33 n = 36 v = 13.59%
Potassium Indirect Potentiometry	x = 5.94 SD= 0.64 n= 10 v =10.77%	x = 7.02 SD= 0.53 n= 14 v = 7.54%	x = 12.85 SD= 1.25 n= 6 v = 9.72%	x = 20.08 SD= 2.35 n= 6 v = 11.7 %	x = 22.65 SD= 2.01 n= 6 v = 8.87%	X = 27.06 SD= 1.72 n= 6 v = 6.35 %	x = 24.06 SD= 3.20 n= 6 v = 13.3 %	x = 25.13 SD= 4.42 n= 6 v = 17.58 %
Sodium Flame Photometry	x = 143.6 SD= 2.5 n=20 v = 1.74%	x = 141.24 SD= 1.94 n= 108 v = 1.37%	x = 136.5 SD= 2.96 n= 36 v = 2.16%	x = 126.55 SD= 3.19 n= 36 v = 2.52%	x = 129.58 SD= 4.37 n= 36 v = 3.37%	x = 123.0 SD= 5.64 n= 36 v = 4.58%	x = 120.36 SD= 6.44 n= 36 v = 5.35%	x = 117.22 SD= 4.54 n= 36 v = 3.87%
Sodium Indirect Potentiometry	x = 140.3 SD= 1.76 n= 10 v = 1.25%	x = 134.57 SD= 1.65 n= 14 v = 1.22%	x = 133.66 SD= 3.44 n= 6 v = 2.57%	x = 126.66 SD= 2.06 n= 6 v = 1.62%	x = 132.50 SD= 5.24 n=6 v = 3.95%	x = 127.16 SD= 4.79 n= 6 v = 3.76%	x = 118.33 SD= 4.41 n= 6 v = 3.72%	x = 113.33 SD= 5.16 n= 6 v = 4.55%

estimated by flame photometry and potentiometry. The Corning 4800 flame photometer was used for the estimation of K^+ ions (the range: 0-150 mmol/L, variability 1.1%) and for Na^+ ions (the range 0-200 mmol/L, variability 0.4%). The biochemical automatic Vitros device was employed for the potentiometric estimation of K^+ (the range 2.5-175 mmol/L, variability 3.6%) and Na^+ ions (the range 5-250 mmol/L, variability 2.7%). The controls and the standards for the Corning 4800 flame photometer and for the biochemical automatic Vitros device were obtained from manufactures. The obtained results were statistically analyzed for the distribution of the results, variance, and differences between particular groups. A normal distribution of data was noted very rarely, and non-parametric tests were employed.

Results

In the smears of vitreous fluid before filtration, stained by H&E, the cells and their fragments were observed at different frequencies in all the studied groups. Moreover, during post-mortem intervals, up to 76 hours, no bacteria were visible in Gram-stained smears. From the 100 hour post-mortem interval, in some Gram stained smears, with different frequency (per microscope field), Gram+ and Gram- bacteria (1-3 per filed) were noted. In all the smears after 124 and 148 hours, bacteria cells were visible, mainly Gram negative. After filtration the smears from LPVF were free from bacterias, cells and their fragments.

A lack of normal distribution of flame photometry results and of potentiometry of both ion concentrations were noted in the studied groups. The results of Mann-Whitney and Kolmogorov-Smirnoff tests showed that significant differences existed between median and data distribution in Na^+ and K^+ levels, derived from the measurement by flame photometry and potentiometry methods in the control and in other studied groups (after 28, 52, 76, 100 post-mortem time intervals), except for the 4 hour and the 128 - 140 hour time intervals. The results of Na^+ and K^+ concentration in the latter post-mortem time intervals revealed a higher variability, as compared to the results in the groups from 28-100 hour PMI. The increase of K^+ concentration in LPVF was proportional to the increase of post-mortem time interval. The decrease of Na^+ concentration in that LPVF was proportional in a similar manner. Such relationships were not dependent on the employed methods. However, the results of the potentiometric measurement of K^+ and Na^+ ion concentrations were significantly lover, as compared to those after flame photometry (Table 1). The increase of K^+ (y) concentration in relation to PMI (x) was not dependent on used method. It was described by two models of regression: y= a + b*sqrt(t) and y= a+b*t, (where "a" is a control level of K^+ concentration, "b" slope per hour (1.98 mmol/L - flame photometry; 2.08 mmol/L - potentyometry). The differences in correlation coefficients and the percentage of explained data were not significant. The concentration of Na^+ ions in LPVF decrease was described by a similar type of equations but: y= a - b*sqrt(t) and y= a - b*t . The first equation was optimal for flame photometry method and the second one - for potentiometry. All the correlation coefficients for all the equations were within the range of 0.86-0.96 with probability p> 0.01

Discussion

The studies on the chemical composition of the vitreous fluid are mainly performed on the eyeballs without removing the organs from the body [4, 6, 7]. Separated eyeballs have been used very rarely and mainly on laboratory animals, e.g., rabbits. The PMI, employed in experimental studies (e.g. pigs, dogs and small laboratory rodents), is limited, in general, to 48 hours post mortem. The presence of Gram minus bacteria in the eyeball and in the aspirated vitreous liquor, established in our studies after 100 post-mortem hours, was not surprising because eyeball sampling was performed in non sterile conditions. Such a bacterial invasion may be a substantial limitation in PMI studies on changes in the eye structure and on the

chemical composition of vitreous body and vitreous fluid. The major advantages of experiments on separated swine eyeballs include: similar weight and age of the animals, veterinary control, identical death, well known time of death and of the collection of organs, very good access to the control of the experimental conditions (e.g. humidity, temperature) [2]. The temperature, at range 6-8^{0}C, is commonly used for the human cadaver storage. In such conditions, as we described earlier, a slower progress of post-mortem changes may better be observed in morphological and immunohistochemical studies [8, 9]. This lowering effect was also visible in our studies on ion concentrations. The changes in K^+ and Na^+ levels in the vitreous fluid, as estimated by us by two different methods, demonstrated a closely related direction. Those changes were in a good agreement with the results of other authors [4, 6, 7]. The differences in the results between flame photometry and potentiometry were described earlier in studies on human vitreous fluid [2]. In the changes of post-mortem K^+ and Na^+ levels in LPVF, two different periods were distinct with a high variability of results. The first period was visible during the first 10 hours post-mortem, the second one - during the last 48 hours of the study. The results during the first period may be dependent on the animal stress [4], an unknown ability of ion binding by gelatinous portion of the vitreous fluid and/or rapid death of ten neural cells, including the autonomic ganglia (interlethal period). The second period may depend mainly on the breaking of the ocular barrier (in situ blood-ocular barrier) by bacterial invasion and storage of the ions by growing bacterial cells. In many publications on the vitreous K^+, determined at post-mortem intervals, the problem of bacterial invasion was not discussed. Our results suggest that bacterial infection participates in the variability of K^+ levels. The influence of bacterial infection on the margin of error for the K^+ post-mortem test remains unanswered and needs further studies. This is very important because such errors can sometimes make this test worthless [2].

Acknowledgments

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