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CASE REPORT

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Hydrocution in a case of Coxsackie virus infection

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Abstract An apparently healthy 7-year-old boy attempted to demonstrate his ability to dive into a whirlpool but was retrieved from the water in a state of unconsciousness after several minutes. Resuscitation was unsuccessful. No characteristic signs of drowning were found at the autopsy but examination of the lymph nodes and the cardiac muscle indicated a pre-existent infection. The histological examination revealed a slight degree of predominantly lymphocytic infiltration of the cardiac muscle. IgM antibodies against Coxsackie virus were detected in the serum sample by means of ELISA. The reverse transcriptase polymerase chain reaction (RT-PCR) performed on an extract of formalin-fixed, paraffin-embedded cardiac muscle tissue revealed a DNA sequence specific for Coxsackie B3 virus. Therefore, cardiac failure was due to a myocardial virus infection and the additional strain caused by diving. This case report emphasizes the importance of modern molecular biological methods in cases of sudden death including death by hydrocution.

Key words Hydrocution · Coxsackie virus infection · Myocarditis · Reverse-transcriptase-PCR · Paraffin-embedded tissue

Introduction

A small percentage of cases of death during immersion in water is due to water-induced reflexes, i.e. hydrocution, socalled “Badetod” in the German literature (Cooke 1993; Knight 1992; Prokop 1975). This definition also includes cases where a pre-existent disorder was present, but was not the primary cause of death (Krauland 1971). In quite a number of cases the disorder seems to be an infection and in these cases it is assumed that death only results in combination with an additional stress occurring under water (Schneider 1992; Schulz 1986). Signs of drowning in these cases may show considerable differences in intensity or may even be missing completely at the time of autopsy (Brinkmann and Püschel 1983; Krauland 1971). Such cases cannot be diagnosed on the basis of autopsy findings alone and additional investigations are always necessary (Reh 1977). The circumstances leading to submersion also have to be taken into consideration. In Germany, this complex of events is of special interest with regard to accident insurance (Brinkmann and Püschel 1983; Krauland 1972; Reh 1977). Modern methods of molecular biology allow a more specific diagnosis in cases of sudden death such as hydrocution, as can be derived from the following case report.

This paper is dedicated to the 65th birthday of Prof. Dr. M. Staak

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Case report

A 7-year-old boy, who was a non-swimmer, jumped into a whirlpool (12 × 5 m, water depth 80–127 cm, 35° C) in order to demonstrate how long he could dive under water. The child had not been observed to have hyperventilated before diving. No paddling or defensive movements were observed during or after submersion. A fellow swimmer retrieved the unconscious boy from the water after several minutes, since he had not surfaced again during this time. Death was certified 2 h later in spite of intensive resuscitation attempts. Any symptoms of illness had not been noticed by his parents or friends before the fatal incident.

Autopsy findings

Body of a boy measuring 125 cm and 25 kg. Petechiae in the skin of the eyelids, conjunctivae and the mucosa. Recent injection marks, an intracardiac injection and superficial injuries with avital aspects in different regions of the body. Greenish tint of the abdominal skin typical for the onset of putrefaction (cadaver age 3 days). No severe injuries in the skin and subcutaneous tissue of the

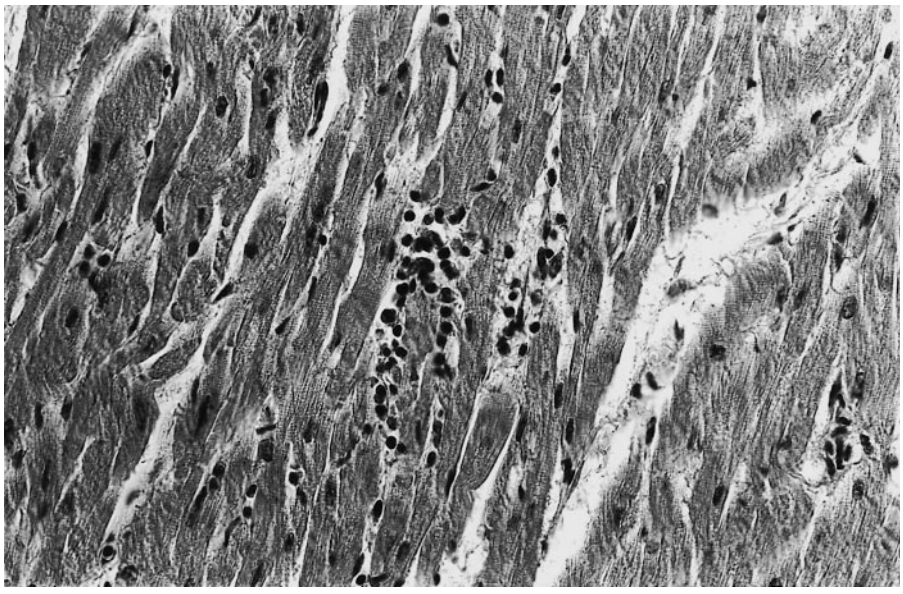

Fig. 1 Myocardium of the left ventricle with a small focus of lymphocytic infiltration (H&E, × 480)

head. Edema of the brain (weight of brain 1503 g). Approximately 120 ml of a slightly hemorrhagic fluid in the pleural cavities. Small amounts of reddish fluid in the trachea, which also showed signs of intubation. Enlarged lymph nodes of the neck. Greyish-brown colour of the cardiac muscle (heart weight 130 g). The lungs of a relatively solid consistency, rich in blood and fluid with no signs of emphysema or diffuse hemorrhages. Spleen with no pathological aspects (spleen weight 84 g). Depleted adrenal glands, 80 ml of largely undigested material in the stomach and a marked enlargement of mesenteric lymph nodes. No pathology aspects of the skeletal muscles or characteristic signs of drowning.

Histological examination

The heart was stored in toto in formalin and samples were examined from all regions. However, no pathology findings could be noted except in a few parts of the left (Fig. 1) and also the right ventricle with isolated discrete but mainly lymphocytic infiltration. A slightly increased number of secondary follicles with activation of the germinal centre was found in lymph nodes. No pathologically significant findings were present in the other organs.

Microbiological investigations

IgM antibodies against Coxsackie virus (B1–3 and B5) were found by means of ELISA applied to cadaver serum. IgM antibodies against Coxsackie virus B4 and B6 were not verifiably positive. No signs of acute or chronic mumps, adenovirus or herpes virus infection were found.

Virus detection by means of RT-PCR

Enteroviral sequences were found in heart muscle tissue which had previously been fixed in formalin (5%, unbuffered) for 60 days and then embedded in paraffin but were not found in neck lymph node tissue. Using the reverse transcriptase polymerase chain reaction (RT-PCR), viral genomic RNA was reverse-transcribed by MMLV reverse transcriptase following the supplier's recommendations (Gibco, Life Science Eggenstein, FRG) with 0.2 mM of primers specific for nucleotides 64-83 or 541-521 of CVB3, respectively (Klump et al 1990). Enzymatic amplification of cDNA was performed as nested PCR on a Perkin-Elmer GeneAmp PCR System 9600 using two 35 cycle programs consisting of denaturation at 94° C for 1 min, annealing at 54° C for 30 s and extension at 72° C for 45 s. Each reaction mixture contained PCR buffer (Perkin Elmer, Norwalk), 1.5 mM $MgCl_2$, 0.2 mM of primers, 200 µM dNTPs/2.5 units of Taq polymerase (Perkin Elmer, Norwalk) to which 2 µl of cDNA reaction mixture or 2 µl of the first PCR product was added, respectively. The outer primers were specific for nucleotides 64-83 and 541-521 of CVB3 (amplification product: 478 bp) and the inner primers were specific for nucleotides 181-200 and 480-460 of CVB3, length of amplification product: 300 bp (Klump et al. 1990). As a control for successful extraction of RNA from myocardial tissue, oligonucleotide sequences were chosen from the cDNA sequence of the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH). Primers were specific for

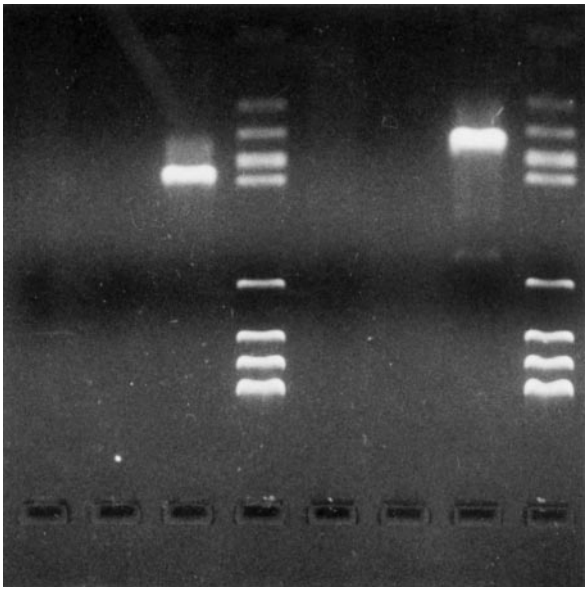

Fig. 2 Measurement of fragment length of the amplification product by gel electrophoresis. From right to left: lanes 1,5: fragment length marker, lane 2: as a positive control for amplifiable RNA of the myocardium, RT-PCR with glyceraldehyde-3-phosphate dehydrogenase mRNA (248 bp), lanes 3,7: in negative controls RNA or cDNA was replaced by aqua dest. in nested RT-PCR, lanes 4,8: PCR with aqua dest. instead of cDNA, lane 6: 300 bp amplification product of CVB3 in the myocardium

nucleotides 3932-3949 and 4355-4372 of human GADPH cDNA (amplification product for mRNA: 248 bp). Measurement of the fragment length of the RT-PCR amplification product resulted in DNA fragments of about 300 bp (Fig. 2). Specificity of amplification products was shown by automatic DNA sequencing and was identified as being Coxsackie virus B3 by means of comparison with known enteroviral sequences (Klingel et al. 1996).

Discussion

In the present case no evidence of drowning was found by the autopsy but the evaluation of findings of drowning would not have been possible in any case due to the preceding intensive resuscitation attempts. On the other hand, it is well-known that resuscitation could lead to multiple petechiae as observed in the present case. The enlarged neck lymph nodes and the unspecific changes in the cardiac muscle tissue suggested the presence of an infection and microbiological investigations indicated a recent Coxsackie virus infection. At the same time, findings of IgM antibodies against several Coxsackie virus subtypes are compatible with known heterotypical reactions (G. Jaeger, pers. commun. 1997). Histological examination revealed no serious findings in heart muscle tissue. According to our experience RNA is degraded faster than DNA by autolysis. However, proof of RNA in autolytic and in archival tissues has been described (Gruber et al. 1994; Jackson and Rintoul 1992). In accordance with Shimizu et al. (1994) enterovirus RNA could be specifically isolated and demonstrated from formalin-fixed material, which was stored for 60 days and this method was applied to verify the presence of a Coxsackie virus B3 infection in the cardiac muscle tissue. Based on this the histological findings could now be interpreted as a cellular activation as usually seen at the onset of myocarditis. However, sudden death cannot conclusively be explained by this infection alone. The contamination with Coxsackie virus can be as high as 80%, whereby most of these infections (up to 90%) are inapparent or cause an undifferentiated febrile illness (Melnick 1996; Modlin 1995). Head out water immersion (33–35° C) leads to a raised cardiac preload followed by a higher cardiac output of up to 50% under thermoneutral conditions (Christie et al. 1990; Weston et al. 1987). It must therefore be assumed that cardiac arrest occurred due to a combination of myocardial virus infection and cardiac strain caused by diving. This case could therefore be classified as a hydrocution (= Badetod). Many reflex mechanisms could be responsible for a collapse of the heart followed by death in water e.g. the Valsalva or Ebecke reflex or laryngeal chemoreflex (Giertsen 1970; Lee et al. 1977; Prokop 1975; Suzuki et al. 1985; Wennergreen et al. 1989). In the present case, reflex mechanisms related to a low water temperature should not be considerable because of the warm water (Giertsen 1970; Missfeldt 1970; Prokop 1975).

The frequency of hydrocution is given as 7% by Missfeldt (1970) and 9.1% by Krauland (1971) of all cases of death occurring in water. About 1600 deaths have happen annually in water in Germany (Statistisches Bundesamt 1997), thus, one would expect about 140 cases of hydrocution.

It seems noteworthy that mainly younger individuals are affected by hydrocution (Missfeldt 1970). They are also overrepresented in the material analysed by Krauland (1971), where in 17 out of 42 cases of hydrocution the affected persons were between 7 and 13 years of age. The present case also belongs to this age group. In adults, alcohol often plays a significant role when hydrocution occurs (Giertsen 1970; Krauland 1971; Reh 1977).

Another point to note is that hydrocution often occurs in non-swimmers and the majority of cases involve children who cannot swim and unexpectedly find themselves in deep water (Krauland 1971; Schwarz 1970). However, these observations cannot be correlated with the assumed reflex mechanisms (Cooke 1993) as vagal reflexes should be present in children, adult swimmers and non-swimmers alike.

Apart from the autopsy, no further diagnostic procedures were carried out in previously published cases of hydrocution and in some cases the results of the histological examination were not given (Krauland 1971; Missfeldt 1970; Raestrup 1972). The importance of additional examinations is also pointed out by the case of a 15-year-old swimmer who died suddenly in water (Schneider 1992). In this case, an infection compromising cardiac function could only be shown after histological and microbiological analyses. It may therefore be assumed that pre-existent states of illness which might have helped in elucidating a number of former cases were not discovered. Complete examinations including microbiological and molecular biological investigations are required in cases of death during immersion in water (Smith et al. 1991) as well as other cases of sudden deaths in childhood (Byard 1997).

As demonstrated by the present case, modern molecular biological methods may aid in the diagnosis of infections, which was not possible in the past (Leparc et al. 1993; Martin et al. 1994). We conclude that the hypothesis of a solely reflectory cause of death in water, as can be proven by animal experiments (Suzuki 1996), is only given in a small number of cases.

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