Response to DDAVP in children with von Willebrand disease type 2

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Summary
We have prospectively evaluated the biologic response to desmopressin (DDAVP) in 28 children with type 2 von Willebrand disease (VWD) in correlation with the phenotype and the molecular defect of VWF. The diagnosis of VWD type 2 was mainly based on VWF functional parameters and/or an aberrant VWF multimer pattern. Seventeen different mutations were identified (6 of them novel). No response with respect to the functional parameters VWF:RCo and/or VWF:CB was seen in patients with severe abnormality of the VWF multimer pattern. One patient with VWD type 2A phenotype IIC Miami did not respond with respect to VWF:CB, but showed a good response of VWF:Ag and FVIII:C as expected. Interestingly he showed a persistently high level of VWF:Ag and FVIII:C up to 4 hours after DDAVP infusion. Patients with minor alterations of multimer structure and particular mutations responded well to DDAVP, whereas patients with normal multimer structure but a defect in platelet dependent functional parameters did not respond to DDAVP. Conclusion: Children with VWD type 2 show a variable response to desmopressin depending on the mutation that correlates with the functional defect and the presence or absence as well as the half-life of large VWF multimers. Our data emphasize the usefulness of DDAVP testing even in patients with VWD type 2, possibly with the exception of VWD type 2B.

Schlüsselwörter
DDAVP, von-Willebrand-Syndrom

Zusammenfassung

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Patients with von Willebrand disease (VWD), the most common inherited bleeding disorder (1, 2), are usually treated either

- stimulating the endogenous release of VWF from endothelial cells with desmopressin (1-desamino-8-D arginine vasopressin, DDAVP) or

- infusion of plasma-derived FVIII/VWF concentrate (3).

DDAVP, an ADH analog, binds to the vasopressin V2 receptor and induces secretion of VWF from endothelial cells by c-AMP-mediated signaling (4). This therapeutic option had been explored initially by P. M. Mannucci (5) and, being an alternative to plasma derived factor concentrates, was later found having decreased the risk of HIV infections of patients with mild haemophilia A and VWD.

However, DDAVP treatment is restricted to responsive patients. Therefore, it is not adequate for patients with VWD type 3 who completely lack VWF. It is regarded as the treatment of choice for patients with VWD type 1. However, recent studies have shown that VWD type 1 is far more heterogeneous than previously thought with respect to

- phenotypes,
- molecular pathomechanisms and
- response to treatment (6, 7, 8, 9).

Therefore, it is generally recommended to test all patients with VWD type 1 for their response to DDAVP and their response duration.

Type 2 VWD, characterized by either decreased, normal or even elevated levels of VWF but a disproportionate decrease of its function has been regarded as non-responsive or of limited responsiveness to DDAVP. However, it could be shown by case reports and by a larger study (10) that DDAVP could be an option for treatment even in cases with functional defects.

Based on these results we adjusted our guidelines for DDAVP testing which since then included patients with VWD type 1 and type 2 to define their individual response.
Due to the potential and actual risk of DDAVP-induced thrombocytopenia we did not include patients with VWD type 2B. We also studied the molecular background of patients with VWD type 2 undergoing a DDAVP test to establish the predictive value of molecular testing for the DDAVP response.

Patients and methods

According to the updated revised classification of VWD type 1, VWD is now defined in a more ‘relaxed’ way (11). This allows to include patients with
- subtle aberrations of VWF multimer structure or
- a relatively slight decrease of large VWF multimers, a feature that previously lead to the diagnosis of VWD type 2A.

In this study we therefore concentrated on patients with a clear aberrant structure of VWF multimers and/or a deficiency of VWF functional activity in relation to VWF:Ag (aberrant ratio). Patients with only subtle aberrations of VWF multimers and their response to DDAVP are reported in detail in the EU funded study MCMDM-1VWD (6, 7, 9).

We studied the response to DDAVP routinely in 28 children above three years of age with VWD type 2 (according to the current classification) of whom we obtained informed consent for genetic testing in accordance with our institution’s ethical guidelines and the amended declaration of Helsinki (Tokyo 2004). Blood pressure, blood count, Na+, Cl– and K+ were determined before and after the test. DDAVP (Minirin®) was administered at 0.3 μg/kg body weight as 30 minutes i.v. infusion diluted in 50 ml of isotonic saline. Between time points 1–2 h and 2–4 h post-infusion, the laboratory parameters studied and their response to DDAVP are part of our standard diagnostic program and illustrated in Table 1. VWF:Ag, VWF:RCo, VWF:CB, FVIII : C, PFA100 closure time and VWF:multimer analysis were carried out as described (12). In order to estimate the duration of the response which is clinically important we also studied a 4 h time point. Response to desmopressin was defined as
- complete if both ristocetin cofactor activity (VWF:RCo) and factor VIII coagulant activity (FVIII : C) increased to ≥ 50 IU/dl,
- partial response as VWF:RCo or FVIII : C < 50 IU/dl post-infusion, but at least threefold the basal level,
- no response as complete lack of either VWF:Ag and/or VWF functional parameters (VWF:RCo, VWF:CB) (9).

Results

Responses to DDAVP

DDAVP was well tolerated in all children, no side effects exceeding flushing were observed. In correlation to the baseline phenotype the response was very heterogeneous.
- Complete response was observed in 17/28 (61%) patients,
- partial response in 2/28 (7%) and
- no response in 9/28 (32%).

Among the latter, one patient with the rare VWD type 2A phenotype IIC Miami, characterized by elevated VWF:Ag but severely reduced VWF functional parameters (13), even showed persistently elevated VWF:Ag and FVIII : C up to four hours but no increase in VWF function (Fig. 1c). However, high molecular weight VWF multimers (HMWM) that usually appear in patients with VWD type 1 after DDAVP were lacking completely. Examples for the different response groups are given in Figure 1 and are described according to the different mutations in detail (Table 2).

Response according to the multimer phenotype

Absence or significant decrease of VWF HMWM were observed in 17/28 (61%) patients. Among eleven patients (39%) with the presence of VWF HMWM three showed an aberrant pattern of individual VWF oligomers (e.g. Fig. 1f) and eight (29%) fulfilled the criteria of VWD type 2M completely, with a decreased VWF platelet dependent functional activity and structurally completely normal VWF multimers (Fig. 1d).

Four patients were deficient in VWF platelet dependent functional activity, showed presence of all size VWF multimers (in three cases even very large HMWM) but had deviations from the normal size distribution of multimers (relative decrease of large HMWM) or a very aberrant structure with multiple intervening bands (Fig. 1f). Twelve patients had a relative decrease of HMWM but relative to VWF:Ag normal functional VWF parameters which suggested a diagnosis between type 1 and type 2. After DDAVP all patients responded with the appearance of larger VWF multimers compared to the initial presentation resulting in a wide multimer response spectrum from persistent severe deficiency of HMWM to complete normalization (Fig. 1).

Response according to VWF mutations

In 8 of the 17 patients with complete response we could identify a mutation. The mutations correlated with
- a moderate phenotype of VWD type 2A, with enhanced proteolysis (IIA, R1597Q; 2435delC) or
- VWD type 2A phenotype IIE with relatively decreased VWF HMWM and an

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Tab. 1. DDAVP-test: schedule for monitoring of diagnostic parameters

<table>
<thead>
<tr>
<th>time</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:CB</th>
<th>FVIII : C</th>
<th>PFA 100</th>
<th>multimers</th>
</tr>
</thead>
<tbody>
<tr>
<td>basal</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
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<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<td>×</td>
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<td>1*</td>
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<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

basal: before the start of infusion; *hours after the end of infusion

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In 9 of the 11 patients with partial or no response we identified one or more mutations that correlated with different phenotypes of VWD type 2A or type 2M (Tab. 2). In one of these patients we identified R1597Q that was also found in the responsive group.

Altogether, seventeen different mutations (6 of them novel) were identified in 14/27 (52%) patients and are detailed in Table 1. In 6 patients with mutations a second mutation seems to be required to explain the phenotype since either only heterozygous quantitative defects (null alleles e.g. 2435delC) or heterozygous recessive qualitative mutations (R202P) were identified. One patient was heterozygous for a gene conversion (P1266Q, V1279I, Q1311X) between VWF and its pseudogene on chromosome 22. Although this gene conversion causes several mutations of VWF, the phenotype is only di-

**Fig. 1** Time course of VWF parameters after DDAVP infusion according to response type, multimer pattern and mutations (if available). 

- **a)** moderately severe VWD type 2A with enhanced proteolysis by ADAMTS13 and partial to good response to DDAVP;
- **b)** severe form of VWD type 2A with enhanced proteolysis and severe impairment of VWF functional activity both of VWF : RCo and VWF : CB;
- **c)** VWD type 2A phenotype IIC Miami (elevated basal VWF : Ag, complete lack of large and medium sized VWF multimers with complete lack of ADAMTS13 mediated proteolytic sub-bands and severely impaired functional activity;
- **d)** VWD type 2M with normal VWF multimers and severely impaired VWF : RCo but normal VWF : CB;
- **e)** VWD type 2A due to compound heterozygosity for the common mutation Y1584C (secretion defect) and the mutation R1374H: significant response according to VWF : Ag but complete lack of response according to VWF functional activity;
- **f)** VWD type 2M with supranormal VWF multimers and numerous intervening bands due to uncleaved VWF-Propeptide, resulting from mutant VWF "Furin cleavage resistance" (mutation R760H) and the secretion defect of Y1584C.
**Tab. 2** Basal laboratory parameters, mutations (novel mutations in bold), and response to DDAVP of patients

<table>
<thead>
<tr>
<th>patient number</th>
<th>VWS type</th>
<th>VWF: Ag</th>
<th>VWF: RCo</th>
<th>FVIII:C</th>
<th>VWF:CB</th>
<th>PFA 100</th>
<th>HMWM</th>
<th>mutation</th>
<th>response to DDAVP</th>
<th>duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>2A</td>
<td>18</td>
<td>15</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td>reduced</td>
<td>no</td>
<td>-</td>
</tr>
</tbody>
</table>
| 02             | 2A       | 84      | 84       | 81      | 40     |         |      | absent   | -                 | complete | sufficient | 2 | 2A | 40 | 7 | 60 | 7 | abs     | - | - | - | complete | short | 2A | 26 | 21 | 39 | 35 | 300 | absent | - | - | - | complete | sufficient | 03 | 2A | 40 | 7 | 60 | 7 | abs     | - | - | - | complete | short | 04 | 2A | 26 | 21 | 39 | 35 | 300 | abs     | - | - | - | complete | sufficient | 05 | 2A | 21 | 7,1 | 34 | 11 | abs     | - | - | - | complete | sufficient | 06 | 2A | 5 | 5 | 6 | 300 | reduced | R202P 605G>C | - | no (1) | 07 | 2A | 103 | 91 | 91 | 300 | reduced | C1153Y 3458G>A | - | complete | sufficient (2) | 08 | 2A | 178 | 6 | 42 | 4 | 230 | abs     | - | - | - | complete | short | 09 | 2A | 13 | 11 | 30 | 13 | reduced | A1105D 3314C>A | - | complete | short | 10 | 2M | 67 | 11 | 111 | 66 | 300 | present | Y1409F 4225G>T | - | - | no | - | 11 | 2M | 35 | 46 | 46 | 46 | 46 | present | Y1409F 4225G>T | - | - | no | - | 12 | 2A | 26 | 10 | 46 | 300 | reduced | R1597Q 4790G>A | - | complete | short | 13 | 2M | 31 | 16 | 80 | 100 | present | - | - | - | complete | sufficient | 14 | 2A | 23 | 7 | 32 | 7 | abs     | - | - | - | partial | ? | 15 | 2A | 18 | 18 | 64 | 100 | reduced | - | - | - | complete | sufficient | 16 | 2A | 47 | 24 | 53 | 30 | reduced | - | - | - | complete | sufficient | 17 | 2A/2M | 13 | 14 | 54 | 300 | reduced | R1315C 3943C>T | - | complete | sufficient | 18 | 1/2A | 28 | 21 | 51 | 28 | 300 | reduced | 2435delC | - | - | complete | sufficient | 19 | 1/2A | 33 | 21 | 69 | 42 | 300 | reduced | 2435delC | - | - | complete | sufficient | 20 | 1/2A | 41 | 30 | 79 | 34 | 221 | reduced | 2435delC | - | - | complete | sufficient | 21 | 1/2M | 59 | 45 | 79 | 52 | 137 | present | - | - | - | complete | sufficient | 22 | 1/2M | 59 | 40 | 86 | 41 | 217 | present | - | - | - | complete | sufficient | 23 | 2M | 42 | 25 | 54 | 33 | 206 | present | - | - | - | complete | sufficient | 24 | 2M | 11 | 9 | 28 | 8 | 300 | present | - | - | - | no | - | 25 | 2M | 13 | 9,5 | 29 | 7 | 287 | present | - | - | - | no | - | 26 | 2M | 20 | 12 | 42 | 17 | 295 | present | - | - | - | complete | sufficient | 27 | 2M | 37 | 28 | 52 | 104 | 300 | present | R760H 2279G>A | Y1584C 4751A>G | - | complete (2) | 28 | 2A | 19 | 10 | 53 | 3 | 300 | absent | G1505R 4513G>A | - | - | no | - | empty cells: no data;  
(1): short duration of individual parameters;  
(2): PFA 100 short correction;  
(3): long lasting correction of individual parameters;  
?: not enough data
rected by Q1311X a nonsense mutation causing a null allele. Compound heterozygosity with a second yet unidentified mutation seems probable.

Four patients were found compound heterozygous: one for the single base deletion 4820delG and the missense mutation M1051T, one for R202P and the presumably non-pathogenic missense mutation P2063S (6), one for R760C and Y1584C, one for R1374H and Y1584C. The previously described R760C alters the consensus sequence of the VWF Furin cleavage site which is necessary to generate the mature VWF protein by cleaving the VWF propeptide from Pro-VWF (14). This mutation causes partial deficiency of Pro-VWF cleavage which results in a very heterogeneous population of differently sized VWF multimers, the presence of very large HMWM and, in the homozygous state, functional deficiency of VWF: FVIII binding. However, the mutation alone does not confer functional deficiency of platelet dependent VWF parameters.

Discussion

DDAVP is considered the treatment of choice for patients with VWD type 1. However, according to recent studies on VWD type 1 a considerable number of patients that were diagnosed with VWD type 1 in the past, had to be considered as type 2 instead (6–8). Additionally, there is increasing evidence that even patients initially diagnosed with VWD type 2 may respond to DDAVP, some partially, some only of short duration but some also completely (10). We therefore collected prospectively the data of 28 consecutive children with VWD type 2 and correlated their response to DDAVP with the respective VWF quantitative and functional parameters, VWF multimer analysis, and the molecular background. We showed that according to generally accepted response criteria (9)

- 61% of patients responded well to DDAVP and
- 39% showed a partial or no response.

In some patients response was of short duration emphasizing the necessity of prolonged response monitoring at least up to the time point of 4 h. The response to DDAVP was consistent in patients with the same mutations although two unrelated patients with R1597Q showed partial response and good response of shortened duration, respectively. Our patient 27 with the mutations R760C/Y1584C showed a good response as did the patient with R760C reported by Casonato et al. (14), however with significantly lower basal parameters suggesting that Y1584C contributes significantly to the phenotype. Patient 17 with the mutation R1315C showed a partial response which is in accordance with published results from another patient with the same mutation (9). A correlation of response with the other underlying mutations can only be speculated on. However, in most cases the results are quite reasonable since the pathogenic mechanism of particular mutations seems plausible and predictive for the response. In conclusion we have shown that a considerable number of patients with VWD type 2 do respond to DDAVP infusions, suggesting that DDAVP testing should generally be considered also in type 2 VWD, possibly with the exclusion of certain patients with VWD type 2B. Extension of such studies will add to a better understanding of the stimulated secretion of VWF which will eventually help to define the optimal therapeutic strategy for the individual patient with VWD.

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