In vitro evaluation of phototoxic properties of systemic antipsoriatics

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Introduction

Systemic antipsoriatic agents such as fumaric acid esters, apremilast, methotrexate, anti-TNF-α agents, ustekinumab and secukinumab induce an amelioration of psoriasis within several weeks, but PASI 100 is not always reached. Phototherapy is superior to certain biologics and induces remission in a shorter time period. Therefore a combination therapy with phototherapy at the beginning of a systemic therapy or in case of only a partial remission can be reasonable. In order to be aware of photosensitive properties of these substances we assessed the in vitro phototoxic potential of these substances using a photohaemolysis test.

Material and Methods

Test substances

Tests were performed with the following compounds:

- Apremilast (Otezla®, Celgene, Summit, USA)
- Adalimumab (Humira®, AbbVie, Wiesbaden, Germany)
- Etanercept (Enbrel®, Pfizer, Kent, United Kingdom)
- Fumaric acid esters (Fumaderm®, Biogen Idec, Ismaning, Germany)
- Infliximab (Remicade®, Janssen Biologics, Leiden, The Netherlands),
- Methotrexate (Metex®, Medac, Wedel, Germany)
- Secukinumab (Cosentyx®, Novartis, Basel, Schweiz)
- Ustekinumab (Stelara®, Janssen-Cilag, Beerse, Belgium)

Benzophenone (Merck, Darmstadt, Germany) was used as positive control.

UV sources

- Waldmann UVA 700 (Waldmann, Schwenningen, Germany), emitting in the region of 340 to 440 nm (maximum about 365 nm).
- TL 20 W/12 light bulbs (Philips, Hamburg, Germany) with a main emission range of 275 to 365 nm (maximum approximately 315 nm).

Photohaemolysis test

Washed fresh human erythrocytes from healthy donors were suspended at a dilution of 1:200 in TCM buffer containing 0.03% human albumin. A 0.4 ml volume of this erythrocyte suspension and a correspondingly prepared erythrocyte-free sample were incubated with 0.1 ml of the test substance preparations at three concentrations (orientated at the plasma concentrations) for one hour at 37°C. Both substance-free erythrocyte samples (blanks) as well as samples containing the test substances were exposed to 0, 25, 50, 75 J/cm² UVA or to 0 (0), 800 (0.36), 1600 (0.72) or 2400 (1.08) mJ/cm² UVB (J/cm² UVA). To obtain total haemolysis (100%) erythrocytes were exposed to distilled water. After an incubation period of 20 min in the dark, supernatants were recovered by centrifugation. The released haemoglobin in the supernatants was determined as cyan-methaemoglobin after incubating the samples for 10 min with Drabkin's solution for 15 min. Haemolysis was determined by reading the absorbance at 550 nm with a Sunrise Microplate reader and calculated on basis of the absorbance data according to the formula:

$$\text{Haemolysis (\%)} = 100 \times \frac{\text{test sample} - \text{blank} - \text{erythrocyte-free sample}}{\text{total haemolysis} - \text{blank}}$$

In order to exclude equivocal results, only haemolysis >5% was regarded as a meaningful positive finding.

Results

Adalimumab induced photohaemolysis up to 8.0% with UVB-rich irradiation. Fumaric acid ester and infliximab induced photohaemolysis up to 13.8 % with UVB-rich and UVA-rich irradiation and ustekinumab up to 11.6 % with UVA-rich irradiation alone (Tab. 1). Single photohaemolysis values of these substances after the highest UV-doses used are shown in Fig. 1 and 2. Apremilast, etanercept, methotrexate and secukinumab did not induce significant haemolysis with UVA or UVB irradiation with values < 5% (Tab. 1).

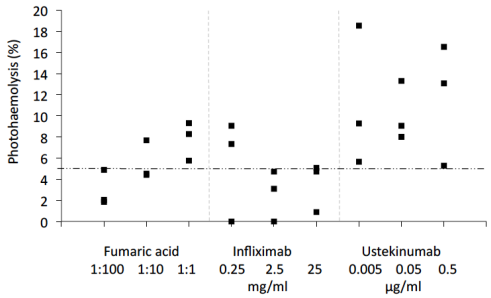

Fig. 1: Single values (n=3) of UVA-induced photohaemolysis (75 J/cm²) by fumaric acid, infliximab and ustekinumab at different concentrations.

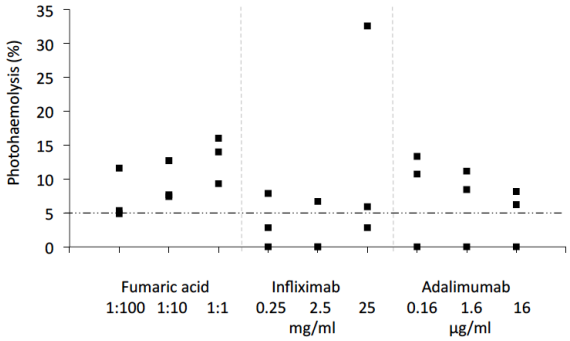

Fig. 2: Single values (n=3) of UVB-induced photohaemolysis (2400 mJ/cm²) by fumaric acid, infliximab and adalimumab at different concentrations.

Tab. 1: Mean (in %) of haemolysis (n=3) induced by apremilast, adalimumab, etanercept, fumaric acid ester, infliximab, methotrexate, secukinumab or ustekinumab and radiation rich in UVA or UVB (bold: values > 5%)

	Not irradiated	UVA 25 J/cm²	UVA 50 J/cm²	UVA 75 J/cm²	UVB 800 mJ/cm²	UVB 1600 mJ/cm²	UVB 2400 mJ/cm²
Adalimumab							
16 µg/ml	0.58	1.41	0.44	1.78	0.13	0.0	4.79
1.6 µg/ml	0.0	1.67	0.25	3.58	0.0	0.0	**6.53**
0.16 µg/ml	0.13	0.38	0.01	3.21	0.13	0.15	**8.02**
Apremilast							
500 ng/ml	0.0	0.0	0.0	0.0	0.75	0.0	0.0
50 ng/ml	0.0	0.0	0.0	0.0	2.51	0.0	0.0
5 ng/ml	0.0	0.0	0.0	0.0	0.38	0.0	0.0
Etanercept							
100 µg/ml	1.31	1.55	2.97	4.12	0.12	0.97	3.04
10 µg/ml	0.67	2.05	1.48	4.03	0.57	0.69	3.23
1 µg/ml	0.0	2.48	1.58	4.76	0.24	0.40	3.25
Fumaric acid ester							
undiluted	1.36	2.78	3.28	**7.78**	0.60	**6.80**	**13.11**
1:10	1.47	1.23	2.51	**5.54**	1.51	**7.33**	**9.27**
1:100	1.37	1.09	0.68	2.86	0.78	**8.00**	**7.28**
Infliximab							
25 mg/ml	0.53	0.01	1.20	3.50	**12.33**	**8.48**	**13.8**
2.5 mg/ml	0.13	2.24	1.30	2.61	1.05	1.32	2.23
0.25 mg/ml	0.10	0.54	0.44	**5.47**	1.31	1.59	3.56
Methotrexate							
10^{-6} M	0.40	1.10	1.63	0.41	1.83	3.71	3.60
10^{-7} M	0.50	0.99	0.81	0.90	0.47	3.72	2.41
10^{-8} M	0.0	1.73	1.21	0.71	0.59	4.77	2.63
Secukinumab							
50 µg/ml	0.0	0.0	0.0	0.0	0.0	0.5	0.0
5 µg/ml	0.0	0.0	0.0	1.37	0.0	0.0	0.0
0.5 µg/ml	0.0	1.01	2.15	0.0	0.0	0.0	0.0
Ustekinumab							
0.5 µg/ml	1.8	0.52	0.67	**11.63**	0.40	0.96	4.63
0.05 µg/ml	2.2	1.48	1.53	**10.13**	0.27	0.92	4.44
0.005 µg/ml	0.69	1.44	0.52	**11.20**	2.10	1.78	4.32
Benzophenone (positive control)							
10^{-3} M	0.11	48.69	85.68	77.40	13.55	32.27	67.00

Discussion

Our in vitro photohaemolysis test demonstrated minor phototoxic properties (< 15% photohemolysis) of some of the used systemic antipsoriatics (adalimumab, fumaric acid ester, infliximab, ustekinumab). Because we did not see increased phototoxicity with either UVA-rich or UVB-rich irradiation the combination of these substances is probably safe with regard to the absence of severe phototoxic properties. Nevertheless acute photosensitizing effects in humans or long-term side effects of such combination therapies cannot be excluded by this test.

References

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