INVESTIGATIONS OF THE SAFETY OF TILDIPIROSIN IN SHEEP

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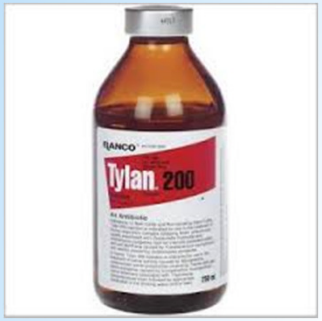
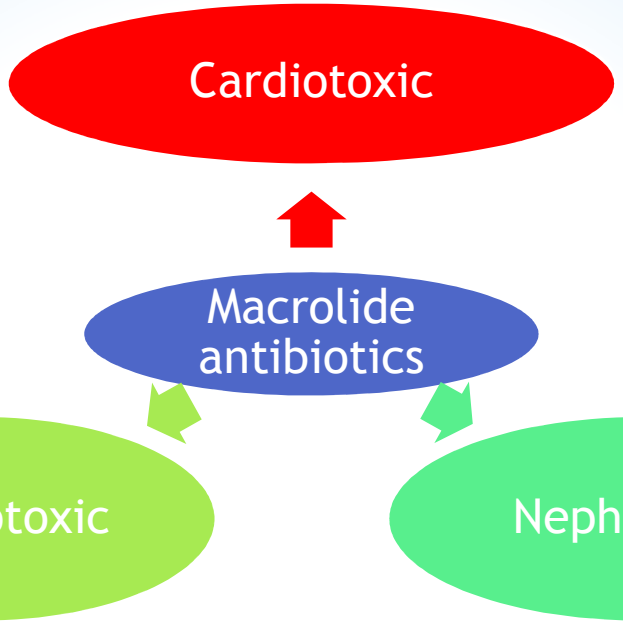
1.Introduction

* Respiratory system diseases have high morbidity and mortality rate.
* *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni* are the group of bacteria recognized as important pathogens,
* And they cause fertility, yield and financial loss in sheep.

- ✓ Currently used antibiotics have been reported to develop resistance against these infectious agents depending on mutations and methylation
- ✓ However, tildipirosin has declared to least resistance developing antibiotics in macrolide group





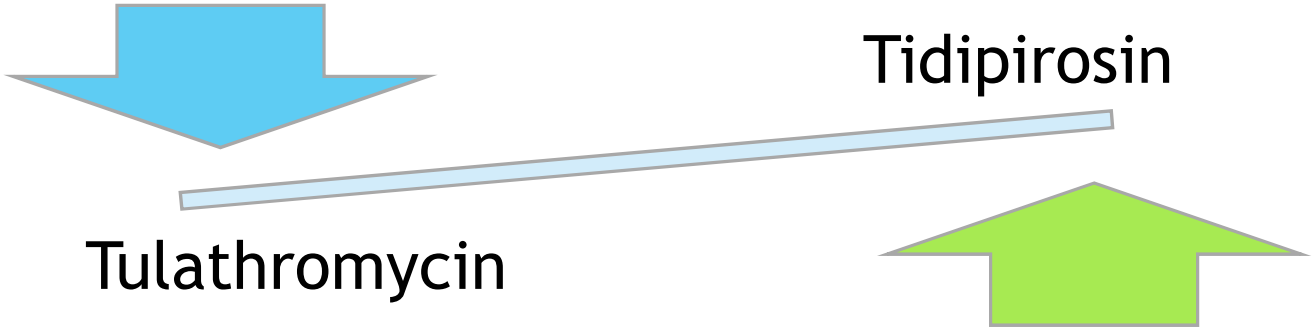
* Especially tilmicosin causes to death in goats
* Also, the drug can cause to death in cattle and sheep
* Tildipirosin is a semi-synthetic-16 membered macrolide antibiotic that is similar structurally to tylosin and tilmicosin
* This antibiotic binds to 50S ribosomal subunit of the bacterial cell wall.

* It has been approved by European Medicines Agency (EMEA) for the use in the treatment of *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* infections at the single dose of 4 mg/kg (SC) in cattle

* Also, tildipirosin uses in the treatment of this infections in pigs

✓ Tildipirosin treats the respiratory infections and

✓ Prevent chronic infectional diseases in transported cattle



- Administration of tildipirosin is more effective than tulathromycin in *Mannheimia haemolytica* induced experimentally lung infection

It has been used as extra label in sheep and other species, although its use in cattle and pigs has been approved by the EMEA

* Though 4 mg/kg (single dose, SC) dose of tildipirosin is safe for cattle, 10 mg/kg (single dose, SC) dose may be cardiotoxic for dogs

* Undesirable effects of drugs in organs and systems can be determined directly via some blood parameters

* Ex : Cardiac damage may be detected by cardiac specific troponin I (TN-I) and creatinine kinase (CK)-MB mass levels

* In the current research, it was aimed that determination of biochemical, hematologic, cardiac and oxidative stress parameters following normal (4 mg/kg, SC) and high dose (8 mg/kg, SC) of tildipirosin used in sheep.

2. Materials and Methods

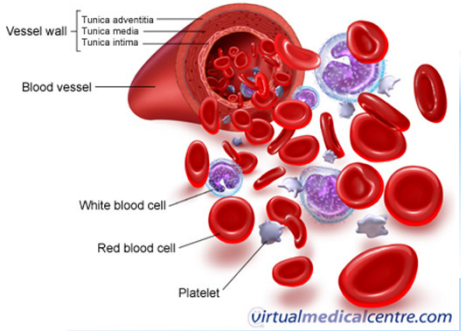
❖Twelve Akkaraman sheep of 4-5 ages, weighing 50-60 kg were used in the study.

In first group (ND) animals received recommended clinical dose of 4 mg/kg (single dose) tildipirosin

The other group (HD) animals were administered high dose of 8 mg/kg (single dose) tildipirosin

•Blood samples were taken before (0. hour, control) and after treatments at on 0.25, 0.5, 1, 3, 5, 10 and 21 days

•Hematology and biochemistry parameters were determined from blood samples



3. Results

✓ Statistically fluctuations were determined in blood urea nitrogen (BUN), glucose (GLC), and CK levels in both group, therefore statistically change was determined in lactate dehydrogenase (LDH) level in ND group ($p<0.05$).

Table 1. Effect of tildipirosin (4 mg / kg, SC) on the serum biochemistry of normal dose (ND) group in sheep (mean ± SE).

Parameters	Day 0 (Control)	Day 0.25	Day 0.5	Day 1	Days 3	Days 5	Days 10	Days 21
ALB (g/dl)	3.0±0.1	3.7±0.2	3.7±0.4	4.2±0.3	4.02±0.2	3.8±0.2	4.1±0.4	3.7±0.3
ALP (U/L)	51.8±12.8	57±12.6	49.7±10.6	50.2±12.6	40±8.3	38.2±8.6	35±9.1	39.2±4.6
ALT (U/L)	17.5±1.9	22.5±1.4	25.5±3.8	30.2±4.1	26.3±6.1	22.8±2.2	21.3±1.8	16.7±1.3
AST (U/L)	74.2±7.6	99.3±5.7	110.3±16.2	133.7±21.6	107.8±13.6	105.5±10.7	117.2±8.3	100.2±7.98
T-BIL (mg/dL)	0.08±0.02	0.1±0.02	0.08±0.02	0.13±0.02	0.15±0.04	0.1±0.02	0.09±0.02	0.08±0.09
BUN (mg/dL)	22.6±1.1^{bc}	32.1±1.8^{a}	27.8±1.7^{ab}	30.2±2.3 ^{ab}	23.4±1.03^{bc}	16.3±0.9^{b}	5.7±1.03^{d}	19.6±1.1^{b}
CHL (mg/dL)	38.8±5.4	48.8±4.6	49.8±7.6	55.0±9.3	52.5±8.6	52.3±2.4	49.3±6.2	41.8±2.2
CK (U/L)	61.0±1.9^{c}	446.5±117.0^{ab}	565.8±62.7^{a}	601.8±137.2^{a}	168.2±11.1^{bc}	162.7±18.5^{bc}	142.3±12.0^{c}	161.5±15.9^{bc}
CRE (mg/dL)	0.7±0.03	0.9±0.06	0.8±0.06	0.9±0.07	0.8±0.04	0.8±0.06	0.8±0.07	0.9±0.06
GGT(UL)	39.0±3.05	47.8±1.5	49.3±5.2	57.2±6.5	50.8±3.6	48.2±1.9	54.8±4.2	58.7±3.9
GLC (mg/dL)	55.2±3.8^{c}	73.3±3.4^{abc}	89.3±7.8^{ab}	99.7±6.9^{a}	64.8±3.7^{bc}	68.5±3.3bc	77.2±2.6^{abc}	84.3±5.4^{ab}
LDH (U/L)	242.5±35.1^{b}	371.8±30.5^{ab}	402.7±73.4^{ab}	483.7±86.9^{ab}	307.2±15.7^{ab}	375.7±68^{ab}	581±48.1^{a}	468.7±44.7^{ab}
TP (g/dL)	6.1±0.4	7.7±0.5	7.7±0.8	8.8±0.8	8.3±0.6	7.9±0.3	8.4±0.7	7.7±0.6
TRIG (mg/dL)	13.7±1.4	14.2±1.2	20±3.02	20.5±4.05	19.7±1.8	20.3±1.05	12.3±1.6	16.3±2.8

ALB: Albumin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, T-BIL: Total Bilirubin, BUN: Blood urea nitrogen, CHL: Cholesterol, CK: Creatine Kinase, CRE: Creatinine, GGT: Gamma glutamyl transferase, LDH: Lactate

Table 2. Effect of tildipirosin (8 mg / kg, SC) on the serum biochemistry of high dose (HD) group in sheep (mean ± SE).								
Parameters	Day 0 (Control)	Day 0.25	Day 0.5	Day 1	Days 3	Days 5	Days 10	Days 21
ALB (g/dl)	3.3±0.3	3.6±0.3	3.5±0.1	3.7±0.4	3.3±0.2	3.2±0.2	3.6±0.1	3.8±0.1
ALP (U/L)	60.8±16.8	50.7±12.5	65.2±12.5	117.5±31.7	75.8±15.08	77.2±12.9	68.3±10.9	87.5±24.9
ALT (U/L)	17.5±1.8	20.0±1.8	21.7±1.7	22.7±4.2	16.5±1.5	19.5±1.8	18±0.9	15.2±1.2
AST (U/L)	94.7±5.7	109.5±10.9	104±7.3	118.8±17.6	94.5±7.1	101.2±7.2	99.8±6.1	103.8±8.6
T-BIL (mg/dL)	0.2±0.04	0.2±0.07	0.1±0.02	0.1±0.03	0.2±0.03	0.1±0.01	0.1±0.03	0.1±0.03
BUN (mg/dL)	23.1 ± 1.5^{a}	25.9 ± 1.6^{a}	24.3 ± 1.4^{a}	22.5 ± 1.1^{a}	20.9 ± 0.6^{ab}	15.6 ± 0.9^{b}	5.5 ± 0.4^{c}	15.9 ± 0.9^{b}
CHL (mg/dL)	56±10.9	59±9.9	50.2±7.4	62±12.4	49.7±8.4	56±4.8	49.7±4.8	43±3.3
CK (U/L)	162.7 ± 18.8^{c}	583.3 ± 145.4^{ab}	806.6 ± 147.4^{a}	313.6 ± 61.0^{bc}	118.0 ± 7.9^{c}	144.2 ± 15.3^{c}	124.8 ± 6.5^{c}	153.5 ± 17.4^{c}
CRE (mg/dL)	0.7±0.03	0.7±0.04	0.7±0.03	0.6±0.05	0.6±0.02	0.7±0.03	0.7±0.02	0.8±0.03
GGT(UL)	49.3±3.7	53.7±5.2	48.8±3.4	52.2±6.6	46.5±3.7	50.7±4.7	51.3±3.5	57.3±4.02
GLC (mg/dL)	65.5 ± 6.3^{b}	76.2 ± 4.5^{ab}	89.3 ± 3.6^{ab}	93.3 ± 6.02^{a}	66.0 ± 2.6^{b}	67.5 ± 3.7^{b}	69.2 ± 4.0^{ab}	81.2 ± 1.8^{ab}
LDH (U/L)	388.2±53.1	448.7±94.8	423±35.2	537±82.03	310.7±29.9	394.2±40.5	512.3±38.4	529.7±57.4
TP (g/dL)	7.6±0.5	8.3±0.9	7.5±0.4	8.3±0.6	7.3±0.3	7.3±0.2	7.8±0.3	8.4±0.3
TRIG (mg/dL)	20.0±2.5	12.5±1.5	19.0±6.1	14.8±2	15.2±1.7	14.2±0.7	12.2±2.3	17.8±2.1

ALB: Albumin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, T-BIL: Total Bilirubin, BUN: Blood urea nitrogen, CHL: Cholesterol, CK: Creatine Kinase, CRE: Creatinine, GGT: Gamma glutamyl transferase, LDH: Lactate Dehydrogenase, TP: Total protein, TRIG: Triglyceride, $^{a.\ b.\ c.\ d}$: Different letters in the same line denote statistical significance ($p < 0.05$).

- ✓ In both groups, other biochemical parameters levels were not determined statistical differences during the experimental period ($p>0.05$).

Table 3. Tildipirosin effects on serum cardiac and oxidative markers on sheep in normal dose (ND) and high dose (HD) group (mean ± SE).								
Parameters	Day 0 (Control)	Day 0.25	Day 0.5	Day 1	Days 3	Days 5	Days 10	Days 21
ND group (Tildipirosin 4 mg/kg)								
CK-MB(mass) (ng/mL)	0.31±0.15	0.41±0.16	0.89±0.21	0.97±0.16	1.50±0.25	1.19±0.44	1.38±0.16	1.10±0.19
Troponin I (pg/mL)	0.015±0.002	0.048±0.030	0.017±0.005	0.009±0.001	0.013±0.004	0.007±0.001	0.003±0.001	0.005±0.001
TBARS (µM)	8.64±2.31	8.69±1.51	5.30±2.51	8.85±0.52	7.00±0.95	11.77±4.63	8.69±2.46	10.05±1.43
HD group (Tildipirosin 8 mg/kg)								
CK-MB(mass) (ng/mL)	0.63±0.20	1.02±0.17	1.33±0.30	1.53±0.13	1.10±0.23	1.05±0.28	0.89±0.27	0.51±0.15
Troponin I (pg/mL)	0.002±0.001	0.071±0.067	0.011±0.005	0.002±0.001	0.004±0.002	0.005±0.003	0.003±0.001	0.007±0.002
TBARS (µM)	3.74±1.39	6.43±1.70	4.25±1.39	2.46±0.60	1.23±1.02	21.53±16.24	47.27±41.49	9.96±3.20

CK-MB: Creatine Kinase-MB, TBARS: thiobarbituric acid reactive substances.
There was no statistically significance in the same line ($p>0.05$)

- The concentration of TN-I in both groups were detected highest on 0.25 days, however statistical difference was not detected between days ($p>0.05$).
- CK-MB (mass) was highest level on 3 and 1 days in ND and HD groups, respectively. However, the statistical change was not detected for CK-MB ($p>0.05$).
- TBARS data have been observed non-statistical significance fluctuations in both groups ($p>0.05$, Table 3).

Table 4. Effects of tildipirosin on hemogram values of in normal dose (ND) and high dose (HD) groups in sheep (mean ± SE).

Parameters	Day 0 (Control)	Day 0.25	Day 0.5	Day 1	Days 3	Days 5	Days 10	Days 21
ND group (Tildipirosin 4 mg/kg)								
WBC (×10⁹/L)	6.40±0.30	8.31±0.77	9.68±1.19	8.13±0.98	7.53±0.76	8.51±0.71	8.45±0.62	9.01±0.90
RBC (×10¹²/L)	9.58±0.65	9.98±0.44	9.95±0.47	9.41±0.44	10.03±0.65	9.98±0.55	11.05±0.82	10.80±0.62
PLT (×10⁹/L)	230.2±17.0	241.3±20.9	222.4±15.3	188.0±12.3	197.8±12.0	225.4±17.0	251.6±11.8	263.3±25.3
HGB (g/dL)	9.06±0.62	9.51±0.32	9.70±0.44	9.70±0.38	10.13±0.58	9.96±0.56	11.15±0.86	11.30±0.67
HCT %	34.40±2.03^{b}	35.65±1.24^{ab}	36.30±1.34^{ab}	34.80±1.12^{ab}	37.43±1.83^{ab}	39.11±1.39^{ab}	43.46±2.74^{ab}	44.48±1.82^{a}
HD group (Tildipirosin 8 mg/kg)								
WBC (×10⁹/L)	7.85±1.01	9.36±1.08	9.50±1.11	8.75±1.27	7.81±0.88	8.88±0.91	9.36±0.46	8.46±0.74
RBC (×10¹²/L)	10.11±0.29	9.73±0.30	9.46±0.17	8.76±0.20	9.48±0.38	9.13±0.33	10.96±0.68	10.51±0.53
PLT (×10⁹/L)	214.6±19.6	175.4±10.7	210.2±21.34	192.0±23.8	206.6±22.4	228.0±21.1	272.2±28.9	244.8±18.7
HGB (g/dL)	9.70±0.41	9.40±0.40	9.95±0.25	9.15±0.46	9.66±0.48	9.03±0.54	10.55±0.90	11.08±0.81
HCT %	37.60±1.72	35.16±1.67	36.83±1.26	33.03±1.94	35.83±2.16	36.25±2.07	42.33±3.28	44.31±3.22

WBC: White blood cell, RBC: Red blood cell, PLT: Platelet, HGB: Hemoglobin, HCT: Hematocrit. $^{a, b}$: Different letters in the same line denote statistical significance ($p < 0.05$).

- ✓ Statistically significant change was determined in hematocrit (HCT) levels in ND and HD groups ($p<0.05$),
- ✓ while white blood cell (WBC), red blood cell (RBC), platelet (PLT) and hemoglobin (HGB) levels did no changed statistically significant between days ($p>0.05$).

4. Discussion

- ✓ Currently used antibiotics may no effective against *Pasteurella*, *Mannheimia* and *Histophilus* pathogens, because these pathogens may show resistance .
- ✓ In this reason, the use of appropriate dosage of new antibiotics such as tildipirosin are important

- ✓ In the current research; clinical dose (4 mg/kg) and double clinical dose (8 mg/kg) have been selected due to tildipirosin has strong dose-response relationship

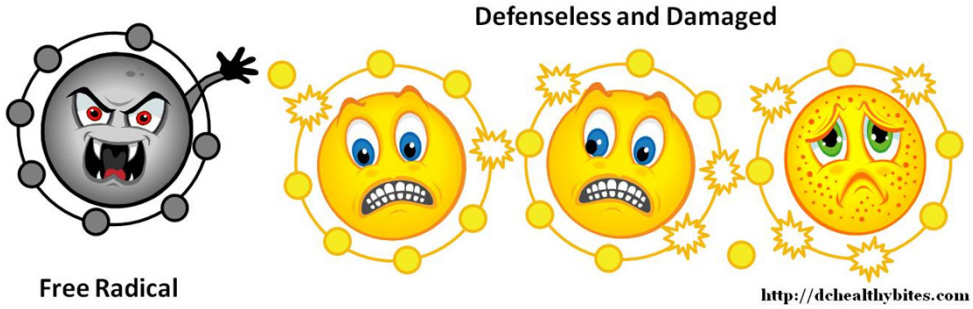
- In the current study, cardiac damage parameters (CK-MB(mass) and TN-I) were fluctuated after tildipirosin treatment in both groups ($p>0.05$, table 3).

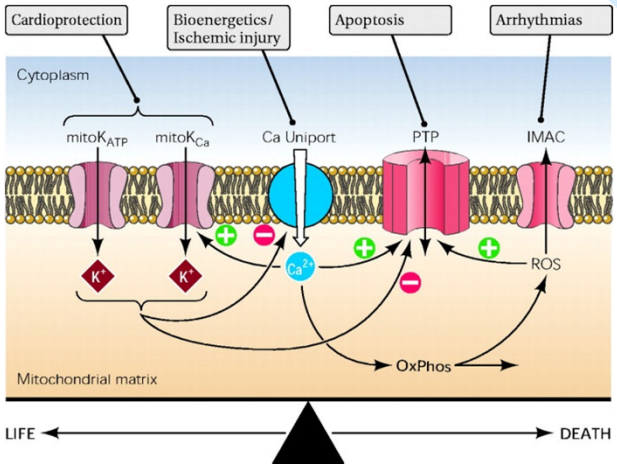
- ✓ Tildipirosin is newly synthesized macrolide antibiotic in recent years, and it may show the cardiotoxicty like other macrolide antibiotics.

- ✓ Tilmicosin has;
  - caused the cardiotoxicity in lamb
  - increase CK-MB levels in mice
  - enhance TN-I and CK-MB levels in rabbits

- ✓ Tulathromycin has reported;
  - to increase TN-I and CK-MB(mass) levels in sheep and rabbits
  - CK-MB and TN-I levels reach the highest level at 24 hours after heart injury

- In the present study, CK-MB(mass) reached the highest level on 3 and 1 days in ND and HD groups, respectively ($p>0.05$).
- TN-I reached peak levels on 0.25 day in both groups.
- Macrolide antibiotics may cause cardiotoxicity and arrhythmia as they increase the permeability in mitochondrial membrane of myocardial cells and levels of free radicals





- In the existing research, tildipirosin may disturb mitochondrial structure of myocardial cells and cause oxidative stress, because it increased levels of cardiac parameters and TBARS ($p>0.05$, Table 3).

- Tildipirosin has been declerated to exist high levels in plasma within 24 hours on pharmacokinetic studies of cattle
  - ✓ Tildipirosin can have altered the permeability of the plasma membrane on 1 day period and
  - ✓ cause the damage especially in the heart and other organs.

- ✓ Liver function and lipid metabolism parameters were within normal range in both groups.
- ✓ Tildipirosin were not change CRE levels in both groups ($p > 0.05$),
- ✓ It increased BUN levels on 0.25 day in ND group and on 1 day in HD group, however these changes were within normal limits in the current research.
- Macrolide antibiotics may cause renal failure due to decrease blood CRE level and change BUN level
- ✓ In this research, tildipirosin treatment may create renal damage in the first 24-hour period; however these findings should be supported by histopathology.

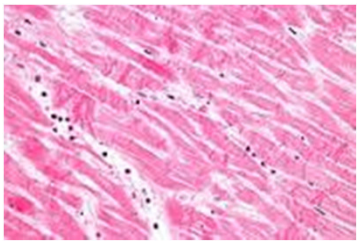
- ✓ In the present study, statistically changes in HCT levels were available in ND group ($p<0,05$), while there were no statistically changes on hemogram parameters in HD group ($p>0,05$)

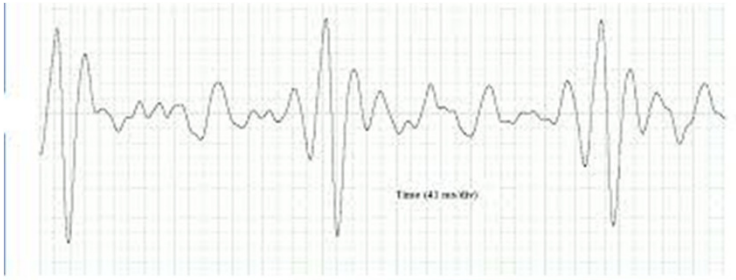
- ➢ Increased RBC, WBC, hemoglobin and HCT and decreased platelet levels have been reported after tildipirosin application by EMEA and FDA

- ➢ It may be stated that tildipirosin application has no significant hematologic side effects in both dose levels at different time points.

5. Conclusion

- ✓ In conclusion, following subcutaneous tildipirosin (4 and 8 mg / kg doses) application may increase in specific blood cardiac and renal damage markers, but not statistically significant.
- ✓ It may cause cardiac and renal damages in both dose levels in sheep.
- ✓ In addition, tildipirosin may cause oxidative stress.
- ✓ However, it may be accepted that tildipirosin has no hepatotoxic and hematologic side effects.
- ✓ For fully investigation of side effects in different dose tildipirosin treatment in sheep, cardiography, monitorizations and histopathological evaluations may be considered in sheep in the future.





Thank you for your patience